

NASA Technical Memorandum 89951

**1986-87 NASA Space/Gravitational
Biology Accomplishments**

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1986-87 NASA Space/Gravitational Biology Accomplishments

Edited by

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*NASA Office of Space Science and Applications
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INTRODUCTION

Preface

An individual technical summary of each research task within the Space/Gravitational Biology Program is presented in this publication. Each summary, prepared by the principal investigator, consists of a description of the research, a listing of the project's accomplishments, an explanation of the significance of the accomplishments, and a list of the publications resulting from the past year's research. Since spaceflight experiments, submitted in response to the Space Biology Dear Colleague letter, have become an integral part of the Program, reports on the activities of the related research are integrated indiscriminately.

Summaries cover the period from January 1986 through April 1987, and highlights from the collective accomplishments are featured as a preamble to the individual reports.

For the first time, the accomplishments of the scientists in the Space Biology Research Associates Program are included in this annual report. The participants in this program, which provides opportunities for postdoctoral scientists to conduct research in the fields of gravitational and space biology, have been outstanding and merit independent recognition.

This publication has two objectives: first, to provide the scientific community and NASA with an annual summary of the accomplishments of the research pursued under the auspices of the Space/Gravitational Biology Program, and second, to stimulate an exchange of information and ideas among scientists working in the Program.

Thanks are due to the Program participants whose research and cooperative response to our requests for information made this report possible. The extensive editorial and technical work provided by F. Ronald Dutcher and Janet V. Powers are gratefully acknowledged and appreciated, as well as the technical assistance provided by April Commodore Roy, Carlos A. Fagundo, and other staff of the George Washington University.

Additional information about this report or the Space/Gravitational Biology Program can be obtained by writing to me at the following address:

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THE NASA SPACE/GRAVITATIONAL BIOLOGY PROGRAM

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Introduction

One of the major features of the physical environment on the surface of Earth is the constant presence of the force of gravity. Terrestrial gravity has important biological consequences for organisms living on Earth. The phenomenon of weightlessness which is encountered on spacecraft provides an excellent biological research opportunity, both because of its uniqueness to space and because of the importance of gravity to life on Earth. Access to space provides an opportunity to manipulate gravity from its norm of one down to almost zero, effectively providing the full spectrum of gravitational research capability for the first time. This capability, combined with the stability and pervasiveness of gravity on Earth, its obvious impact on biological evolution, and its continuing effect on the morphology, physiology, and behavior of living organisms, has led the Space/Gravitational Biology Program to concentrate its efforts and resources on investigating the biological significance of gravity and advancing knowledge in the biological sciences through the use of the microgravity environment of spaceflight.

Program Goals

The goals of the Space/Gravitational Biology Program are to: use the unique characteristics of the space environment, particularly microgravity, as a tool to advance knowledge in the biological sciences; understand the role of gravity in the biological processes of both plants and animals; and understand how plants and animals are affected by and adapt to the spaceflight environment, thereby enhancing our capability to use and explore space.

Program Scope

Research in the Space/Gravitational Biology Program is divided into four broad areas:

1. Gravity Perception/Sensing. Plants and animals have developed gravity-sensing systems that facilitate orientation and locomotion within Earth's environment. The weightless environment of space provides a unique research opportunity to understand how gravity-sensing systems of different organisms have developed, and how they process and transmit information. Current research

includes the investigation of the mechanisms of gravitropism and how plant growth is selectively regulated, and how animal gravity sensors integrate and process sensory information. The reconstruction and modeling of the functional organization of gravity sensors is expected to lead to increased understanding of how information is processed by biological systems.

The goal is to understand gravity-sensing systems in plants and animals, the role gravity plays in their development and evolution, and how they are affected by weightlessness. Specific objectives are:

- a. To identify gravity-sensing organs and mechanisms , and to define how they function and adapt to weightlessness.
- b. To understand how gravitational information is transduced, processed, transmitted, and integrated into a response.

2. Developmental biology. Research in this area examines how gravity, and especially the microgravity of spaceflight, affects genetic integrity, cellular differentiation, development, growth, maturation, and senescence, and how it influences life span, reproduction, and subsequent generations.

The goal is to understand the role and influence of gravity on the processes of reproduction, growth, development, and subsequent generations.

Specific objectives are:

- a. To determine if organisms and multiple generations of organisms can develop normally in microgravity.
- b. To identify gravity-sensitive developmental stages, systems, and mechanisms in both plants and animals.
- c. To understand the effects of gravity and weightlessness on gravity-sensitive developmental stages, systems, and mechanisms.

3. Biological adaptation. All biological species on Earth have evolved under the influence of gravity. In response to this force, organisms have developed structures to withstand gravity loads, as well as regulatory systems to function optimally in the terrestrial gravity level of 1 g. The biological adaptation program conducts research examining the physiological effects of altered states of gravity in various plant and animal species. This includes the use of gravity's physiological effects to explore biological problems; understanding how gravity affects and controls the physiology, morphology, and behavior of organisms; how gravity and other environmental stimuli and stresses interact in this control; and the biological mechanisms

by which living systems can respond and adapt to altered gravity, particularly that of the space environment.

The goal is to identify and understand the systems and mechanisms living organisms have evolved in adapting to Earth's gravity, and how they are affected by microgravity. Specific objectives are:

- a. To understand the influence of gravity on the evolution, regulation, and function of biological support structures.
- b. To determine the role of gravity in regulating metabolism, metabolic rate and products, fluid dynamics, and biorhythms.
- c. To understand basic mechanisms of mineral and hormonal homeostasis and the role of calcium as a mediator of gravitational effects.
- d. To identify the effects on living organisms of the interaction of environmental factors (e.g., temperature and light) with gravity, and determine the mechanisms involved.

4. Cell Biology. Cells that are building blocks of systems (e.g., plant root caps), individually functioning units of certain tissues (e.g., blood cells), and unicellular organisms (e.g., paramecia) have been shown to be sensitive to gravity. Research focuses on how gravitational loading influences cell functions and the molecular mechanisms regulating them. Microgravity is an essential tool in testing this hypothesis.

The goal is to determine the mechanisms of gravity's influence on cellular and molecular functions. Specific objectives are:

- a. To investigate the role of gravity in maintaining normal cellular and molecular function.
- b. To distinguish direct from indirect, extracellular, or systemic, gravitational effects on cells.
- c. To discriminate between the influences of cosmic rays, microgravity, and other environmental factors.
- d. To assess the permanence of effects on cells exposed to microgravity.

Focus of Program

The current Program is focused on answering the following basic scientific questions:

1. What are the components of the gravity-sensing mechanisms of plants and animals? How do they sense gravity and how is the information transduced, processed, transmitted, and integrated to evoke

responses?

2. Does gravity influence fertilization and development of plants and animals, and can fertilization and development proceed normally in a near-zero gravity environment? If gravity does have an effect, what are the sensitive developmental stages and physiological systems and how are they affected? If early development is affected by gravity, is it a result of an effect on the parent or on the embryo itself?

Many of these questions can be answered about plants when a controlled seed-to-seed experiment is conducted in space. This has been a long-standing program objective. Supporting ground-based and flight research are considered to be of top priority because of their scientific value and contributions to the Controlled Ecological Life Support System (CELSS) program.

3. What is the role of gravity in the formation of structural elements such as lignin, cellulose, silica, chitin, and bone calcium phosphates at the molecular level as well as at more complex organizational levels?
4. What role does gravity play in calcium-mediated physiological mechanisms and in calcium metabolism?
5. How does microgravity influence cell functions and structure?
6. What role does gravity play in regulating metabolism and metabolic rate and products, and what are the effects of weightlessness?
7. How does gravity as an environmental factor interact with other environmental factors to control the physiology, morphology, and behavior of organisms? Or, how do gravitational and other environmental stimuli interact in the control and direction of living forms? Can the action of gravity be replaced by different stimuli?

Research Opportunities

While the research supported and encompassed by the Space/Gravitational Biology Program is primarily ground-based, space flight experiments are an essential component of the program.

Spaceflight provides the validation for experimental hypotheses developed in ground-based research, while gravitational experiments on Earth will continue to hone the questions, provide the necessary baseline data, and develop spaceflight experimental protocol.

The experimental approach of the ground-based studies in the Program is to manipulate gravity on Earth and develop weightless simulation models to: (a) develop and test gravitational hypotheses, (b) identify gravity-sensitive biological systems and interacting environmental response mechanisms, (c) analyze biological systems and mechanisms known to be gravity-sensitive, (d) analyze flight experiment data and iteratively expand ground research capability, and (e) plan and design future space experiments. In addition, research is conducted to understand how the uncontrollable biodynamic factors of the spacecraft will affect the results of the various flight experiments.

The Space Shuttle is currently the only U.S. developed spacecraft capable of carrying biological experiments. Two avenues are available to propose Shuttle flight experiments through NASA. An Announcement of Opportunity, "Life Science Investigations in Space 1986-91," formally solicits proposals. The next proposal due date is February 1, 1989 with selection to be announced in March 1990.

Unsolicited proposals for flight experiments received in response to a 1983 Dear Colleague letter on "Emerging Opportunities in the Space Biology Program" have been accepted on an ad hoc basis since the announcement. This opportunity, created for biological experiments to be flown in the Shuttle Orbiter mid-deck on a space available basis, was closed temporarily after the Challenger accident but should become available again in 1988.

The limited opportunities to conduct biological experiments on spacecraft have stimulated the examination of alternative means to conduct space research. Biosatellites offer such an alternative. While they require an increased level of automation beyond the "mid-deck" experiment approach, they have the significant advantage of extended stay in space. With strong support from the scientific community, Biosatellite experiments could be flown in 1990.

The research of the Space/Gravitational Biology program is dependent upon several dynamic factors: the requirements of NASA, the characteristics of flight experiment opportunities, the sensitivity of specific biological systems to gravity, the scientific value of the research, the state of knowledge and technology in the specific scientific areas, the interest of scientists in studying the biological questions, and the availability of funds to support the research.

ACCOMPLISHMENT HIGHLIGHTS

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GRAVITATIONAL/SPACE BIOLOGY
ACCOMPLISHMENTS HIGHLIGHTS

PLANT

- o Measurements of electric current changes across plant organs show that electric/ionic currents play a key role in gravity perception.
 - Current changes occur in roots at the precise locus of gravity sensing and with the same timing as is required for completion of sensing.
 - These changes can be inhibited with inhibitors of calmodulin, but not by auxin inhibitors. This implicates a role for calcium ion movement in gravity sensing.
 - Application of a small electrical potential, which makes the plant shoot tip positive, drastically reduces growth.
- o Membrane channels which pass increased numbers of ions in response to stretch and other factors may serve as the mechanism in plants for the mechanotransduction of gravity and other physical forces.
 - A second stretch-activated channel, this one a cation channel, has been found. An anion channel was identified previously.
- o Additional evidence for and against amyloplasts as the gravity sensor has been obtained.
 - The only place in dark-grown stems where sedimented amyloplasts are consistently found is in the region of the stem that bends in response to gravity.
 - The ability of a mutant of the Arabidopsis plant to respond to gravity, even though it does not contain starch (which comprises the bulk of amyloplasts), has been confirmed.
 - Shoot and root cells which contain sedimenting amyloplasts possess a network of microfilaments. This is the first demonstration of microfilaments in these cells. Microfilaments surround both the nucleus and the amyloplasts.

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- o Calcium has been shown to play a key role in the sensing and/or transduction steps of gravitropism. Recent research indicates:
 - Calcium, calcium-dependent protein phosphorylation, and inositol phospholipid turnover have all been linked to transduction of the gravitropic signal in roots.
 - A nucleoside triphosphatase (a nuclear enzyme involved in messenger RNA synthesis) was purified and shown to be stimulated by calcium-activated calmodulin. This is the first demonstration of a nuclear enzyme regulated by calcium and calmodulin.
 - Aluminum ions have effects opposite from those of calcium ions in terms of root curvature, calmodulin activity, and auxin movement. This evidence supports the calcium-calmodulin-auxin model.
 - The calcium gradient that results from movement of calcium across gravitropically responding roots is most pronounced in the mucilage and cell wall. Removal of the mucilage does not affect the response, however.
- o A protein which binds to auxin transport inhibitors called phytotropins has been partially characterized. This protein may be part of a mechanism regulating auxin transport.
- o Some roots when grown in the dark cannot respond to gravity unless illuminated. In these roots:
 - The level of calmodulin, an enzyme regulated by calcium, increases fourfold in root caps within 30 minutes of illumination.
 - Some forms of vitamin D can induce gravitropic responsiveness in these roots. Vitamin D is known to induce calmodulin synthesis in plants.
 - Serotonin also can induce graviresponsiveness. Serotonin is known to activate in animals a second messenger system called the phosphoinositol-trisphosphate system, which regulates the calcium content of cytoplasm.
 - Graviresponsiveness can be restored by very low levels of red light, levels which can be recorded quite deep in soil.

- o Mutants offer considerable promise in understanding the effects of gravity on plants.
 - More than 100 gravitropic Arabidopsis mutants have been identified.
 - One of these mutants has altered shoot gravitropism but normal root gravitropism, which indicates at least one critical element differs in shoot and root gravitropism.
 - A tomato mutant which does not respond to gravity appears to have an auxin transport or auxin receptor defect.
- o The bending response in gravitropism is now known to be based upon different rates of cell wall expansion on different sides of the root or shoot.
 - A series of monoclonal antibodies have been raised against cell wall antigens, including two directed against a wall peroxidase and two against a wall cellulase. These peroxidases and cellulases have been confirmed to be present in the wall.
 - The pattern of metabolites found in the lower half of gravistimulated shoots (decreased starch, increased glucose) is consistent with enhanced cell wall synthesis there.
 - Cell wall loosening during plant growth is much more dynamic than previously thought, increasing severalfold within a few minutes.
 - The epidermal cell layer of roots must be present for a gravity response to occur.
 - During gravitropic bending of cereal shoots, parenchyma cells just inside the epidermis on the upper side become visibly compressed. Epidermal cells do not show this compression.
- o Reduced growth in inverted plant stems has been linked to increased glycoprotein and lignin production and inhibition of cell elongation.
- o Calcium has been implicated in growth inhibition due to mechanical perturbation.
- o Centrifuge studies indicate that the hypergravity of launch may have been the cause of the damping, arrhythmicity, and increase in period length observed in a Neurospora circadian rhythm experiment conducted on STS-9.

ANIMAL

- o In mammals, gravity is sensed by the vestibular system. Recent progress includes:
 - The first 3-dimensional reconstructions of a weighted neural network were obtained.
 - Work with these reconstructions demonstrates that mammalian gravity receptor cells process information sent to the central nervous system in parallel, not serially. They act, therefore, as an information network.
 - A key ion transfer enzyme is elevated in specific regions of one chamber of the vestibular system, the utricle, but not in the other chamber, the saccule, supporting the notion that fluid balance in the saccule is maintained from outside the saccule chamber.
- o Gravity sensing systems in less complex animals are providing useful information about gravitational information transduction and gravity receptor organs.
 - Electrical signals were shown to increase in an invertebrate during gravitational stimulation. Ion channels involved in the increase are selectively permeable to sodium ions.
 - The first direct recordings of a single ion channel from any graviceptor neuron were obtained. The recordings show the presence of a potassium channel that is open much of the time.
 - In birds, mineralization of developing gravity receptor crystals occurs gradually, with intermittent periods in which the process seems to be accelerated. At least three different biochemical moieties have been demonstrated in the organic component of the gravity receptors.
- o Research into the effects of gravity on animal cells and fertilized eggs has demonstrated:
 - Specific amphibian egg cytoplasmic constituents, which rearrange during gravity orientation following egg fertilization, were shown to possess differing mobilities. The mobilities are unrelated to the density of yolk packing.

- Cultured amphibian nerve and muscle cells exposed to a continually changing gravitational vector exhibit morphological and functional changes. Cellular metabolism is slowed.
- Reorientation of mammalian eggs relative to gravity affects chromosomal movement, expressed as an inhibition of cell division during meiosis.
- Pituitary cells, which are the source of growth hormone, were shown to be altered in growing rats exposed to spaceflight, i.e., they contained more hormone but released less of it into the blood. Ground-based mechanically unloaded rats showed a similar defect.
- o Muscles atrophy and bone demineralizes when the force of gravity is not present. Research into the basic biological processes underlying these phenomena has shown:
 - Muscle tension partially protects against a bone growth defect in bones that are mechanically unloaded.
 - Muscles atrophied due to mechanical unloading show an increase in sensitivity to the hormone insulin. The changes occur rapidly (within 4 hours), but also rapidly return to normal with reloading.
 - The blood supply to mechanically unloaded bones appears to increase in growing rats.
 - The hinge portion of the lower jaw in growing rats is very sensitive to spaceflight.
 - Bone-degrading cells appear to remove bone mineral first and then to also degrade the bone matrix which holds the mineral.
 - The active form of vitamin D appears to stimulate the development of bone-degrading cell precursors.
 - When the active form of vitamin D is kept at a constant level in growing rats, the bone appears less mature than the bone in normal rats.
- o Research into the effects of gravity on other systems of animals has shown:
 - Growing rats show a significant delay in adapting to changes in gravity, e.g., body temperature and temperature biorhythms are depressed for days after increased gravity and do not synchronize with the light/dark cycles as do normal Earth rats.

- The site of metabolic development of a critical brain locus for biorhythms was different in rats born and raised in a 2 g field as compared with 1 g rats.
- Several types of ion channels were identified in brain tissue so that electrical channel currents could be simulated to provide information on the contribution of each channel to the brain response to incoming signals.
- A portion of the brain, the hypothalamus, was found to be more prominent in regulating fluid and electrolyte balance than had previously been suspected.

FLIGHT

- o Ground-based research and development in support of flight experiments continued during the past year.
 - Design of variable g-force middeck centrifuges for plant experiments has been completed.
 - Fabrication and testing of Gravitational Plant Physiology Facility (GPFF) flight hardware for plant experiments continued.
 - Biocompatibility studies between flight hardware and yeast and jellyfish test organisms were conducted.
 - Yeast strains have been improved, including discovery of a yeast mutant which can be physically separated by fluorescence, enormously speeding mutant isolation.
 - Hardware, materials, and procedures preparation for Neurospora circadian rhythm experiment continued.
 - Development of test systems for plant cell culture experiment continued.
 - Materials and protocols for sea urchin development experiment were refined.
 - Methodology was developed and ground-based studies conducted for a jellyfish experiment. An inflight Chemical Delivery System was developed.

PLANT PROJECTS

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AN ATTEMPT TO LOCALIZE AND IDENTIFY THE GRAVITY-SENSING MECHANISM OF PLANTS

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Description of Research

Our objective is to solve the problem of how gravity is perceived and transduced at the cellular and molecular levels. To accomplish this we have established and defined a plant system in which the gravitational stimulus is rapidly transduced into an asymmetric distribution of the plant growth hormone, indole-3-acetic acid (IAA).

Briefly stated, the working hypothesis we have developed is: (a) a change in the plant's orientation, with regard to the Earth's gravitational field, causes a perturbation in the plant's bioelectric field; (b) the change in the plant's bioelectric field opens and/or closes voltage-gated channels between the plant's vascular tissue and the surrounding cortical tissues; (c) the resultant altered movement of hormone, calcium, and potassium causes asymmetric growth; and, (d) normal metabolism of the hormone, calcium, and potassium soon results in resumed normal symmetric growth.

Accomplishments

(1) Analytical chemistry: We acquired a new gas chromatograph-mass spectrometer. With the aid of this instrument and Dr. Jerry Cohen's new ^{13}C -labeled IAA standard, we have attained an analytical precision for IAA of 0.1%. Thirteen different samples of plant cortex material, grown and harvested over a 13-week period, yielded values for free IAA of 9.79 ± 0.89 nanograms per gram. Thus, our analytical variability plus the plant variability has been reduced to 9%. No bioassay, or previously developed physicochemical assay for IAA is comparable in precision, certainty of identification and, usually, in sensitivity. This has important application to planned future spaceflight experiments.

(2) Electrophysiology: We have developed a method for applying a voltage, from top to bottom of the plant, which in terms of voltage drop from cell to cell is comparable to the plant's endogenous electrical potential. We have demonstrated that making the tip of the plant positive, with an applied potential of only 0.6 mV per 10 μ cell reduces growth by about 90%, whereas that same potential with the tip of the plant negative causes no inhibition of growth. The knowledge that small applied potentials can cause drastic changes in growth rate

is consistent with our theory that plant gravitropic responses may, in fact, be responses to gravity-induced changes in the plant's bioelectric potential.

(3) Enzymology of IAA metabolism: Once the IAA has moved out of the vascular stele and into the surrounding cortical cells, it is subject to both oxidation and to conjugation. Studies of the enzymes catalyzing both these processes are in progress in this laboratory. To correctly estimate the IAA moving from stele to cortex, we must have measurements of both these processes. Both the enzyme catalyzing conjugation of IAA to form IAA-glucose and the enzyme oxidizing IAA to oxindole-3-acetic acid have been extracted and partially characterized.

(4) Physiology of IAA transport: Little is known about how IAA and its conjugates move from seed, through the vascular stele, and into the surrounding cortical tissues. We have completed a series of studies showing that IAA may be loaded into the stele and then selectively moved into the cortex in response to a gravity stimulus. We are developing methods to identify and to ultimately isolate the channels through which the IAA moves from stele to cortex.

Significance of the Accomplishments

(1) Analytical Chemistry: Studies of gravitational physiology should be brought to the cellular and molecular level to understand the complex biomedical events that occur in a microgravity environment. To this end, a reductionist, and simple, gravity-responsive system is necessary, and we have developed such a system. We have focused our attention upon highly sensitive and accurate measurement of chemical changes.

(2) Electrophysiology: If the basic working hypothesis we have developed is correct, that is, that small electrical perturbations can induce large growth changes, then it may be possible to use small fixed fields to help orient the growth of plants in a microgravity environment, such as that of the space platform.

(3) Enzymology of IAA Metabolism: Knowledge derived from the above experiments and from flight experiments will improve terrestrial agriculture by helping to understand plant hormonal homeostasis.

(4) Physiology of IAA Transport: This has agronomic implications and may solve problems of cell to cell movement in plant and animal tissues.

Publications

Bandurski, R.S. and Ehmann, A. GC-MS Methods for the Quantitative Determination and Structural Characterization of Esters of Indole-3-Acetic Acid and myo-Inositol. In: Modern Methods of Plant Analysis, Volume III (ed. by H.F. Linskens and J.F. Jackson). Heidelberg: Springer-Verlag, pp. 189-213, 1986.

Bandurski, R.S. and Schulze, A. Movement of Indole-3-Acetic Acid from Stele to Cortex of Zea mays Seedlings (Abstract). Plant Physiology 80 (4, Suppl.): 26, 1986.

Bandurski, R.S., Schulze, A., and DesRosiers, M.F. Possible Effects of Organelle Charge and Density on Cell Metabolism (Abstract). In: Twenty Sixth Plenary Meeting of the Committee on Space Research, Abstracts. Paris: COSPAR, p. 313, 1986.

Bandurski, R.S., Schulze, A., and Reinecke, D.M. Biosynthetic and Metabolic Aspects of Auxins. In: 12th International Conference on Plant Growth Substances (ed. by M. Bopp). Heidelberg: Springer-Verlag, pp. 83-91, 1986.

Lewer, P. and Bandurski, R.S. Occurrence of 7-Hydroxy-Oxindole-3-Acetic Acid in Seedlings of Zea mays (Abstract). Plant Physiology 80(4, Suppl.): 95, 1986.

Nonhebel, H.M. Measurements of the Rates of Oxindole-3-Acetic Acid Turnover, and Indole-3-Acetic Acid Oxidation in Zea mays Seedlings. Journal of Experimental Biology 37: 1691-1697, 1986.

Reinecke, D.M. In vitro Oxidation of Indole-3-Acetic Acid to Oxindole-3-Acetic Acid by an Enzyme System from Zea mays (Abstract). Plant Physiology 80(4, Suppl.): 118, 1986.

Reinecke, D.M. and Bandurski, R.S. Auxin Synthesis and Metabolism. In: Plant Hormones and Their Role in Plant Growth (ed. by P. Davies). Netherlands: Nijhoff, M./Dr. W. Junk, Publishers, 1987.

RESEARCH IN GRAVITATIONAL PLANT PHYSIOLOGY

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Description of Research

Our long-range research goals are to improve our understanding of how plants acquire and use gravitational information for directing the course of morphological development and physiological behavior. Unique access to the protracted microgravity environment of spaceflight makes the scientific exploitation of Shuttle flight opportunities the most important operational objective of our research efforts.

Short-range goals include:

- (1) To implement by design, fabrication, and qualification of flight hardware the objectives of GTHRES, a candidate flight experiment for the International Microgravity Laboratory (IML)-1 Mission.
- (2) To implement by design, fabrication, and qualification of flight hardware the objectives of FOTRAN, a companion experiment, also a candidate for flight on the same IML-1.
- (3) To implement objectives of the proposed AMYSED experiment, which will require development of in-flight centrifugation facilities more sophisticated than those sponsored by NASA for previous biological studies.

Accomplishments

(1) After some attempts by experienced personnel of the Jet Propulsion Laboratory data enhancement laboratory, we concluded that further efforts to unscramble the still uninterpretable body of data from SL-1 HEFLEX flight experiment video tapes would be futile. Therefore we have written a full scientific report of HEFLEX, and a manuscript is being submitted for publication.

(2) Preliminary design phase of AMYSED flight hardware has been completed. The instrumentation consists of a paired set of two, independently operated, variable g-force centrifuges sized to fit a Shuttle Middeck Double Locker. When developed, the unit not only will be used to implement our AMYSED experiment, but should be useful for other investigators whose experiments may require onboard centrifugation.

(3) Development efforts continued for the fabrication and testing of Gravitational Plant Physiology Facility (GPPF). This consists of flight hardware for two separate experiments which will measure phototropic responses of Triticum shoots (FOTRAN) and gravitropic responses of Avena coleoptiles to graded stimuli by blue light or by centripetal forces (GTHRES). Plant responses to those stimuli will be measured as they occur in the absence of a significant background g-force. The two experiments are related only by the flight hardware within which they make common use of various facilities. Thus, GPPF is a unit even though the investigator teams (which overlap) are not identical. GPPF has been included as a candidate experiment for IML-1. In March 1987, an Investigators Working Group (IWG) was designated by NASA; a projected launch date (20 April 1990) was announced; and Payload Specialist selection was authorized to proceed.

(4) In connection with the collection of ground-based data on phototropic kinetics of Triticum responses to illumination, we were concerned with the possible increase in tungsten lamp filament temperature when the lamps are required to operate in zero g instead of 1 g. Short exposures to near weightlessness can be achieved in parabolic trajectories of subsonic aircraft. Two KC-135 test series were conducted successfully. The increased light output attributable to absence of thermal diffusion was on the order of + 5%, so the effect can be easily accommodated for the FOTRAN experiment which requires a controlled photic stimulus in microgravity.

(5) For the GTHRES experiment, it will be important to suppress mesocotyl growth of the Avena test seedlings, since it is only the coleoptile whose response should be measured. By a series of test exposures in flight-type apparatus ("Mesocotyl Suppression Box"), a set of red light intensities and exposure times were related to the measured effectiveness of mesocotyl growth suppression. From the results of those tests, we were able to establish the growth stage and red light flux that would be optimal for producing the best pretreated (mesocotyl-suppressed) test plants for use during the orbital mission.

(6) In collaboration with scientists from the University of Trondheim, Norway, we completed a set of centrifugation experiments to explore further the influence of graded hypergravity conditions on parameters of Helianthus circumnutation.

Significance of the Accomplishments

Finding #1. The scientific reports on the HEFLEX experiment project will provide the customary documentation of a definitive test of a scientific theory that could be tested adequately only in a microgravity environment. The test was successful. The

results changed the way most plant physiologists explain the plant behavioral function called circumnutation.

Finding #2. The AMYSED middeck locker centrifuges not only will be essential for implementing objectives of the AMYSED experiment, but they should be useful for other investigators whose experiments may require onboard centrifugation during spaceflight.

Finding #3. The FOTRAN and GTHRES experiments should provide for the first time quantitative data on responses of plant shoots to light and to g-forces when those responses are not complicated by a persisting background of Earth's gravity.

Finding #4. The physical effect of gravity on light production by a heated filament in a gas-filled tungsten lamp bulb is dependent on convection within the bulb, a phenomenon already known to most physicists but, to our knowledge, not easily predicted quantitatively from theoretical considerations. In our case an empirical measurement of this effect was important since light intensity must be a controlled variable in the FOTRAN experiment. Also, it seems not unlikely that, given some numbers with appropriate statistical treatment, some engineers and physicists may find our data useful in ways not closely related to plant physiology.

Finding #5. Development and use of the "Mesocotyl Suppression Box" are essential to obtain the best results from the GTHRES Avena experiment. On the ground, photosuppression of mesocotyl elongation is a standard procedure more easily achieved without the constraints of a spaceflight experiment. Without mesocotyl suppression the growth response to be measured by GTHRES would have two major components -- mesocotyl elongation (and curvature) and coleoptile elongation and curvature. It is only the latter that we seek to measure in GTHRES.

Finding #6. Ten years ago we explored the g-force dependence of circumnutation by a particular cultivar of Helianthus annuus and found it nearly insensitive to incremental changes in centripetal force above unit g to more than 10 g. At that time the favored theory could not easily accomodate those results, a theory which in other respects had been able to predict the behavior of a different cultivar of H. annuus. It seemed appropriate to be able to compare the behavior of both cultivars in hypergravity by the same methods and apparatus. We found that there was a quantitative difference in the circumnutational behavior of the two cultivars--not surprising, but a warning that it often can be a mistake to generalize from results obtained on only one genetic strain of a test organism.

Publications

Dahl, A.O. Observation on Pollen Development Under Gravitationally Controlled Environments. Linnean Society Symposium "Pollen and Spores - Form and Function", PP. 44-59, 1986.

MICROGRAVITATIONAL EFFECTS ON CHROMOSOME BEHAVIOR

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Description of Research

The long-range goal of this research project is to further understand the evolutionary role of gravity on cell division through the study of the effects of space environment on genetic processes. The specific objectives of the research are: (a) to determine the effects of microgravity and space radiation on chromosomal structure, recombination, and transmission during both mitotic and meiotic cell division, (b) to discriminate between effects due to microgravity alone and those due to space radiation, and (c) to relate these findings to the process of sexual differentiation and gametogenesis. To accomplish these objectives, a simple eukaryotic organism, the yeast Saccharomyces cerevisiae, is employed as a cellular model system. Various strains of yeast, constructed "ad hoc" by conventional genetic and recombinant DNA technology, will be incubated in small growth chambers filled with different types of media and flown aboard the Space Shuttle, inside the Biorack incubator. Part of the cultures will be placed inside a centrifuge able to reproduce the same gravitational force present on Earth. At the end of the mission, the cultures will be retrieved and subjected to further genetic and ultrastructural analysis.

During the past year, several basic aspects of the research have been conducted in the laboratory. These scientific aspects are a fundamental part of the overall preparation for the flight experiment. The construction of the yeast strains has been almost completed and some of the strains have been used to test a new system able to detect rare mutational events on the basis of the color of the cells, using the Fluorescence-Activated Cell Sorter (FACS). One of these strains, a diploid hybrid, has been employed to perform biocompatibility studies on the flight hardware developed by NASA Ames Research Center. Other experiments consisted of hypergravity stress analysis of yeast chromosomes. Finally, we have started to clone a yeast gene, the presence of which confers stability to the chromosomes during cell division. Since one of the possible targets of microgravity and/or space radiation is the machinery responsible for chromosomal stability, the basic knowledge of the elements of this machinery will be beneficial in interpreting the flight experiment data.

Accomplishments

(1) Yeast strain construction. We have completed the construction of two more strains that will be employed during the

spaceflight experiments.

(2) Studies have been performed using live cells to determine the biocompatibility of the flight hardware developed by NASA. This hardware consists of plastic growth chambers fitted with movable pistons to allow the injection of a fixative solution into the chamber itself. Cell viability, genetic stability, and differentiation are the parameters considered to assess the hardware's biocompatibility. At the end of these tests the flight hardware has been found biocompatible with the yeast cells.

(3) Hypergravity studies. We have previously found a lethal effect of 10 g gravitational acceleration on haploid strains but not on diploids. Recently we have proven that hypergravity per se does not induce mortality but that this effect is probably due to vibration stress. We are therefore now investigating this aspect with the use of an accelerometer, a device able to measure and quantitate mechanical vibrations.

(4) Fluorescence-dependent analysis of mutations. In the course of our investigative effort to improve postflight analysis, we have discovered that adenine biosynthetic yeast mutants which accumulate a red pigment exhibit primary fluorescence. This fact has been exploited by the use of the Fluorescence-Activated Cell Sorter to physically separate rare mutant cells from normal ones. The significance of this system is that one can isolate mutant cells with a speed enormously higher than today's standard genetic techniques.

(5) The cloning of the chromosomal-stability gene cdc6 is in progress. We have purified a genomic yeast DNA library obtained from another laboratory and begun the transformation experiments to select the clones to be screened.

Significance of the Accomplishments

Results from experiments of group 1 provide us with the yeast strains necessary to perform the genetic tests in space and on the ground. Results from experiments of group 2 are important for the preparation of the flight experiment.

Results from experiments of group 3 have ruled out the lethal effect of low-g centrifugation on single cells. They also have narrowed down the potential cause of cell mortality to the effects of vibrational stress.

Results from experiments of group 4 have confirmed our previous preliminary data on the possibility of using the FACS system to quickly screen large populations of cells to identify particular mutants. Because the same mutants existing in yeast are known to exist in mammalian cells, we think that the same mutational analysis is possible in mammalian organisms. If this is true, it could provide a unique way to detect radiation damages of human genes in live white blood cells.

SHOOT INVERSION INHIBITION OF ELONGATION IN THE RELEASE OF APICAL DOMINANCE

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Description of Research

Branching patterns and plant form are determined to a large extent by apical dominance, the control exerted by the terminal bud over the outgrowth of lateral buds. That lateral bud outgrowth in Japanese Morning Glory can be induced by inversion of the upper shoot provides a promising approach for elucidating the interaction between gravity and the control mechanisms of apical dominance.

Our long-term objective has been to determine how shoot inversion-induced gravity stress in the inverted shoot causes the release of apical dominance in the highest lateral bud of Japanese Morning Glory. Our more immediate objective has been to determine precisely how the gravity stress of shoot inversion inhibits elongation of the inverted shoot and to elucidate the possible mediating role of ethylene and of other factors which may be involved in this process. Biochemical analyses of effects of shoot inversion and of ethylene on certain components and properties of cell walls in the growing region of the shoot have been carried out. Evidence for possible cross linking associated with glycoprotein formation has been investigated. Specific determinations have been made on the content/activity of hydroxyproline, peroxidase, phenylalanine ammonia-lyase (PAL), phenol and lignin. Anatomical studies on the effects of gravity stress of shoot inversion on cell size, shape and character have also been performed.

Accomplishments

(1) The production of cell wall hydroxyproline in the inverted shoot is promoted by the gravity stress of shoot inversion within 12 hr. That this response is mediated by ethylene is suggested by the observation that this increase in hydroxyproline content in the inverted shoot was largely negated by treatment with AgNO_3 , the ethylene action inhibitor, and that hydroxyproline content in the upright shoot was increased by ethylene treatment.

(2) Cell wall peroxidase activity was increased 32 to 63% and 55 to 66%, respectively, by inversion and ethylene treatments over a 12- to 24-hr period.

(3) Phenylalanine ammonia-lyase (PAL) activity was dramatically stimulated by shoot inversion and ethylene treatments, especially after 24 hr. Inhibition by AgNO_3 was small but significant.

(4) After 24 hr, total phenol content was also enhanced by inversion and ethylene treatments. AgNO_3 significantly negated the inversion- and ethylene-induced production of phenol.

(5) Shoot inversion and ethylene treatments increased lignin content by 30% and 38%, respectively, after 24 hr. AgNO_3 partially negated these increases in lignin.

(6) Preliminary qualitative evidence for the presence of isodityrosine in the inverted stem tissue was found.

(7) Anatomical observations (made in collaboration with Dr. Fred Sack) indicated that shoot inversion caused a decrease in internode and cell length in the growth region of the stem. In the swollen portion of the stem immediately behind the growth region there was an increase in cell diameter in all tissues (pith, vascular, cortex and collenchyma) (Figure 1). The shape of the cortex cells was mostly isodiametric in the upright shoots whereas it was mostly rectangular in the inverted shoots.

Significance of the Accomplishments

Findings #1 through #6 are consistent with the hypothesis that hydroxyproline-rich glycoprotein (HRGP) and lignin play a role (via some cross-linking mechanism) in the later stages of gravity stress-induced cessation of elongation in the inverted shoot. Other workers have suggested that wall-bound peroxidase may catalyze the insolubilization of HRGP in the wall via cross linkage of isodityrosine. This presumably decreases the wall extensibility and restricts growth. Lignin has also been shown by other investigators to be associated with cessation of growth. PAL is the first enzyme in the phenylpropanoid pathway which provides phenolic compounds, the early precursors of lignin. Peroxidase is known to polymerize the final precursors of lignification.

The anatomical studies (Finding #7) suggest that shoot inversion-induced restriction of elongation in the growth region of the shoot is due to inhibition of cell elongation and not due to inhibition of cell division. The swelling of the inverted stem behind the growth region is mostly due to increased cell size rather than to increased cell number via enhanced longitudinal cell division.

That gravity stress-induced ethylene may play a role in mediating (a) the production of glycoprotein and lignin, and (b) the restriction of growth in the inverted shoot is of particular significance in assessing the possible effects of ethylene which may emanate from plants under the microgravity conditions of spaceflight.

Publications

Prasad, T.K. and Cline, M.G. Kinetics of Shoot Inversion Induced Ethylene Production in Pharbitis nil. Botanical Gazette 147: 437-442, 1986.

EFFECT OF INVERSION ON PLANT STEM AND CELL SIZE

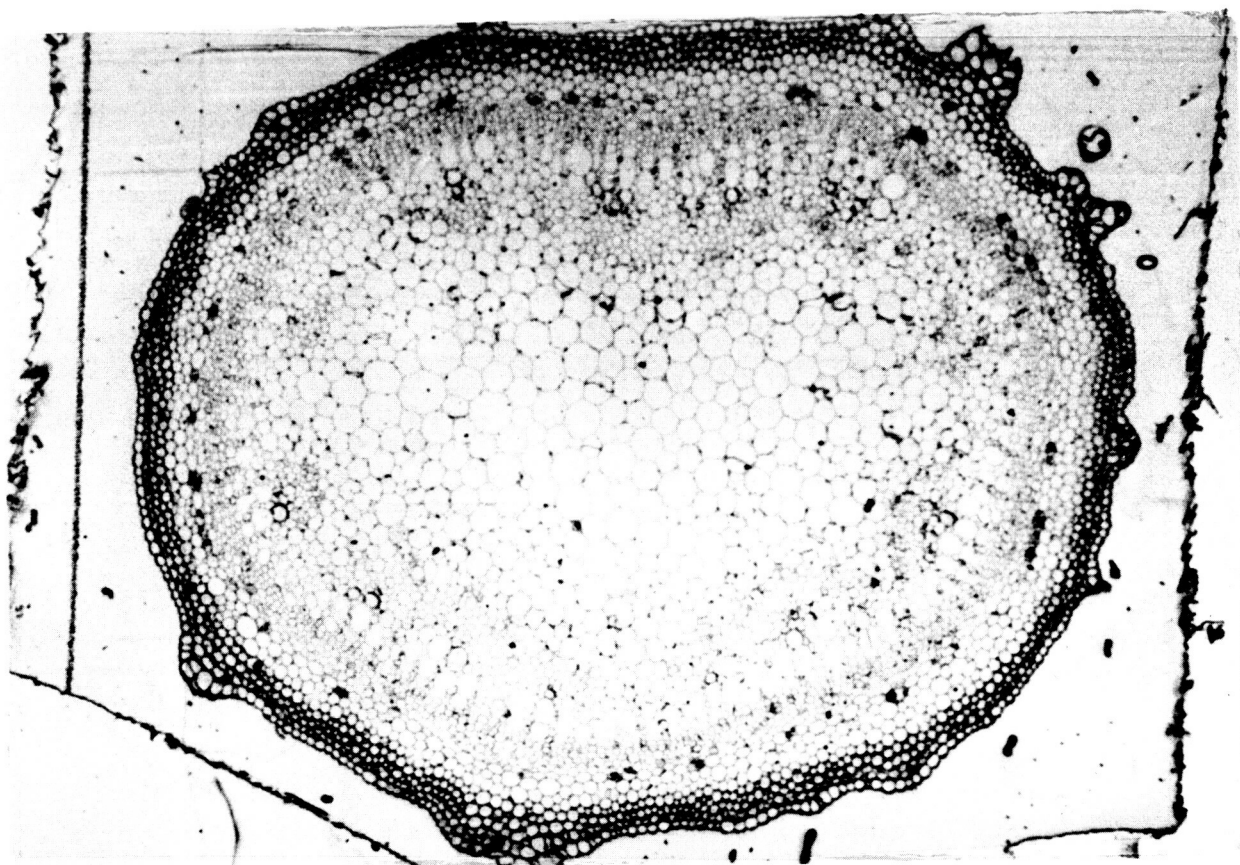
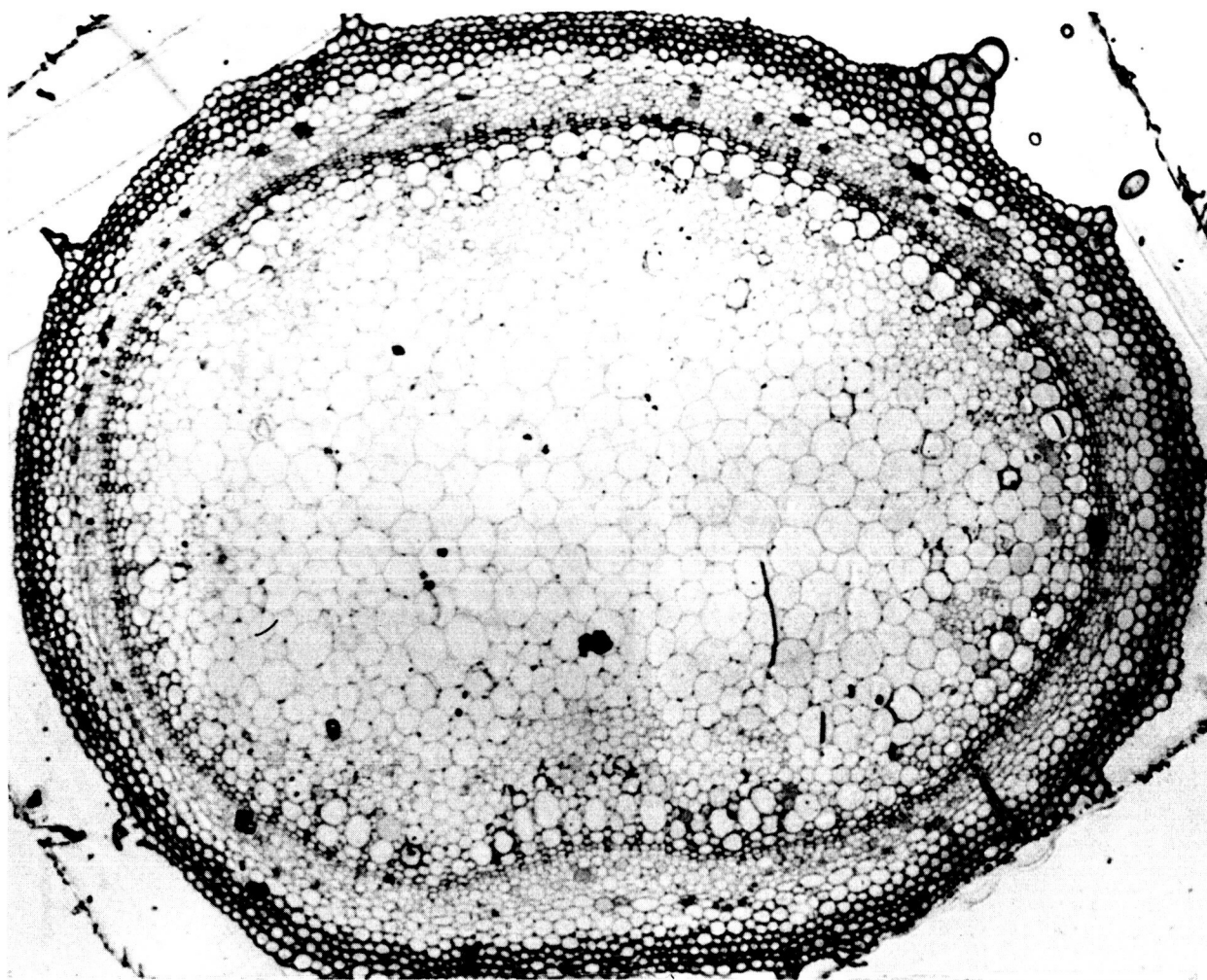


Figure 1. Above: Cross section of upright Japanese Morning Glory stem after 72 hr. Facing page: Cross section of stem after being inverted for 72 hr. The sections were taken at a position which was marked 2-3 cm behind the stem tip at zero time. Note increased diameter of stem and cells in inverted stem.

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Prasad, T.K. and Cline, M.G. Restriction of Elongation in Inverted Pharbitis nil Shoot: A Role for Hydroxyproline? (Abstract). Plant Physiology 80(4, Suppl.): 114, 1986.

Prasad, T.K. and Cline, M.G. The Control of Apical Dominance: Localization of the Growth Region of the Pharbitis nil Shoot. Journal of Plant Physiology 125: 185-190, 1986.

Prasad, T.K. and Cline, M.G. The Role of Gravity in Apical Dominance: Effects of Clinostating on Shoot Inversion-Induced Ethylene Production, Shoot Elongation and Lateral Bud Growth. Plant Physiology 83: 505-509, 1987.

MECHANISM OF DIFFERENTIAL GROWTH DURING STEM GRAVITROPISM

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Description of Research

This project is part of our long-term goal to elucidate the biophysical and cellular mechanisms controlling plant cell growth. Gravity has both subtle and dramatic effects on plant growth. We are studying the rapid alteration of growth which occurs when a plant stem is turned on its side. Our previous work with cucumber seedlings showed that stems bend upwards by ceasing growth on the upper stem surface and doubling growth on the lower surface. This growth asymmetry results in upright growth of the stem. It occurs rapidly (lag of less than 10 min) and opposing growth responses occur in cells within 1 mm of each other (across the stem diameter).

What is the mechanism for the simultaneous, local modification of growth on the two sides of the stem? The aim of our work in the past two years has been to characterize and quantify the biophysical basis for these changes in growth. Cell enlargement entails both water uptake and irreversible wall expansion. We are examining how these two physical processes are interrelated and are altered during gravitropism.

Accomplishments

(1) We have modified and improved the pressure microprobe, to permit greater flexibility and ease for making measurements of the internal cell pressure (turgor pressure) of individual growing cells. The new method uses a video detection system in combination with a microcomputer to automate some of the pressure microprobe functions.

(2) We have measured the turgor pressure of cells on the lower and upper sides of the bending cucumber stem. The results confirm and extend our previous finding, that the changes in growth are not caused by changes in turgor pressure. Our recent results show a slight decrease in turgor on the lower (faster-growing) side, and a slight increase in turgor in the upper (slower-growing) side. The changes are about 0.2 bar, on a baseline turgor of about 4 bar.

(3) We have devised a new method for measuring cell wall yielding properties by in vivo stress relaxation. The technique, termed "pressure block", allows high temporal resolution of the dynamics of cell wall yielding.

(4) Our pressure-block experiments with the cucumber stem show that wall yielding is much more dynamic than previously believed. Within 2-5 min of blockage of cell expansion with the pressure-block apparatus, the growing cells increase the

wall-loosening process by 2 to 5 fold.

(5) We have constructed and tested calcium-selective microelectrodes. They have shown Nernstian responses within the range of pCa 1 to 5, and in some cases are stable for as long as 2 days (i.e., long enough to carry out multiple experiments).

Significance of the Accomplishments

Finding #1. A major redesign of the pressure microprobe was necessary because of technical difficulty in measuring cell turgor pressure in a bending stem. The new design is much easier to use and for some measurements, such as "pressure clamp" measurements, it greatly improves resolution.

Finding #2. This finding leads to three conclusions. First, the alteration in cell growth under the influence of the gravity vector is not caused by altered turgor. The changes in turgor are in the wrong direction for them to cause the altered growth rate. It is most likely that the slight turgor alteration is a secondary consequence of the altered growth rate (water influx rate). Second, the turgor pressure results mean that stem gravitropism is principally the result of altered cell wall properties. No evidence for altered water transport characteristics was found. Third, the internal water potential gradient sustaining water influx into the expanding cucumber stems is small -- perhaps 0.5 bar. This follows from the observation that the turgor changes were so slight, whereas the growth (water influx) changes were quite large.

Finding #3. A major problem with earlier techniques for measuring wall properties by in vivo stress relaxation was that growing tissue had to be excised to isolate it from a water supply. This limitation is now overcome with the pressure-block technique. Furthermore, this new method can be used with a wider variety of tissues than is currently possible with the pressure microprobe method.

Finding #4. The dynamic increase in wall loosening in the first few minutes of the pressure-block procedure was surprising, and not predicted by current theories of plant cell growth regulation. Cucumber appears to possess an extreme or exaggerated form of growth regulation which also exists, in a less remarkable way, in other plant species. At present we do not understand the basis for this growth regulation, since it implies a complex control mechanism only hinted at in earlier studies.

Finding #5. Results from other laboratories implicate an important role for wall calcium in the gravitropic response of plant stems and roots. However, quantitative measurements of the activity and amount of calcium in the wall are conspicuously lacking. We are beginning efforts to measure wall calcium and to investigate its role in plant growth regulation. We plan direct tests of current hypothetical ideas about changes in wall calcium

during gravitropism.

Publications

Cosgrove, D.J. Mechanism of Growth Inhibition in Water-Stressed Pea Seedlings (Abstract). Plant Physiology 80(4, Suppl.): 104, 1986.

Cosgrove, D.J. Photomodulation of Growth. In: Photomorphogenesis in Plants (ed. by R. Kendrick and G. Kronenberg). Amsterdam: Martinus Nijhoff/Dr. W. Junk, pp. 341-366, 1986.

Cosgrove, D.J. and Durchko, D.M. Automated Pressure Probe for Measurement of Water Transport Properties of Higher Plant Cells. Review of Scientific Instruments 57: 2614-2619, 1986.

Cosgrove, D.J., Ortega, J.K.E., and Shropshire, W., Jr. Pressure Probe Study of the Water Relations of Phycomyces blakesleeianus Sporangiohores. Biophysical Journal 51: 413-423, 1987.

Cosgrove, D.J., Ortega, J.K.E., and Shropshire, W., Jr. Water Relations of Phycomyces Sporangiohores (Abstract). Plant Physiology 80 (4, Suppl.): 90, 1986.

Dunford, S.A. and Cosgrove, D.J. Gibberellin Control of Stem Elongation in Pea Seedlings (Abstract). Plant Physiology 80(4, Suppl.): 116, 1986.

SEEKING THE MECHANISM OF GRAVITY DETECTION IN ROOTS: THE ROLE OF PLASMALEMMAL ION CHANNELS

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Description of Research

The gravitropic response of roots of many plants serves to provide support for the aboveground portion by anchoring the plant in the Earth and thereby enhancing the probability of access to a source of minerals and water therein. The response, an altered growth pattern orienting the root positively with respect to the force, is initiated upon detection of a change in gravitational field relative to the growing axis of the root and entails an increasingly complex set of modulating factors. Although a large number of factors have been identified as ostensible mediators of the response, neither the mechanism of gravity detection nor the chemical and electrical sequences of events resulting in curvature are known. However, one of the first molecular steps must be the rapid modulation of membrane ion channels leading to the measured changes in membrane potential, proton and calcium fluxes, and changes in external current patterns along the root. My current research focus is to examine directly the channels present in plasma membranes of cells thought to contribute to gravitropic response of roots, the outer and inner cells of the root cap, the meristem, and the epidermal and subepidermal cells of the elongation zone.

I am presently visiting Dr. Stan Misler's lab in order to learn the mechanics and method of patch-clamping, the approaches used in the study of ion channels in animal cells, and to establish the best productivity from my patch-clamp equipment. I have built a voltage stimulator for my patch-clamp system and a quantitative device for studying stretch-activated channels (SAC). With this basis I am developing techniques to easily patch plant cell protoplasts to investigate ion channels in plasma membranes and to identify those with potential function in gravitropism.

The patch-clamp method is an electrophysical technique for high-resolution recording of the activity of individual ion channels in membranes of intact cells or in isolated membrane fragments (or in artificial membranes) as described in 1985-86 NASA Space/Gravitational Biology Accomplishments. The technique can be used to characterize the activation, selectivity, gating, and metabolic as well as second messenger modulation of channels.

Channels are activated or inactivated (opened or closed) by one of four mechanisms: membrane voltage, transmitter (neurotransmitter or transported hormone), intracellular modifier

(e.g., calcium, ATP, calmodulin, c-AMP), or sensory energy (e.g., mechanical, such as membrane stretch, or physical, such as light). All of these mechanisms may operate channels critical to the gravitropic response. The most rapid changes in some cells following gravitropic stimulation include membrane depolarization and hyperpolarization, changes in proton and calcium flux, and perhaps some hormone alterations. All of these events could modulate channel activities and result from channel activity at the plasma membrane and intracellular membranes. But gravitropism must begin with the detection of the physical stimulus of gravity, which could be potentiated by sensory-regulated channels. In this category, the recently discovered group of stretch-activated channels (SAC) appears as a very logical candidate. In the previous edition of this publication, Barbara Pickard and I discussed the possibilities such channels might hold for the detection and transduction of various physical stimuli in plants important to gravitropism as well as osmoregulation, cell division, and morphology. Lee Falke and I have been the first to show that SAC exist in plant cell membranes (Falke et al., 1986). Evidence for SAC in plants came from large cultured tobacco stem cell (gift of Monsanto) protoplasts released following removal of the cell wall by enzymatic means. The anion-selective SAC was found in both intact protoplast and excised membrane patches (Fig. 1A). SAC have now been found in yeasts (Gustin et al., 1987), and they are appearing widespread in animals. This evidence enhances expectations that SAC will be found in roots.

This year, while learning the patch clamp technique, I have been methodically working out the parameters needed to acquire plant protoplast patches easily and consistently, since our success rate was very low (one useful one per week, or less). The two publications to date (Moran et al., 1984 and Schroeder et al., 1985) describing channels in plasmalemma of higher plant cells from patch-clamp data also indicate that these membranes are difficult to patch. Second, I have eliminated the need to anchor protoplasts with polyethyleneimine, an undesirable protocol used in our initial attempts to patch tobacco protoplasts. Caution arises from the fact that the related molecule, polylysine, does effect membrane permeability. Third, SAC of the tobacco cell protoplast have been further characterized for publication, and fourth, the identification and characterization of SAC in the putative gravity sensing cells, the columella of the corn root cap, has been initiated. The immediate goal of this work is to characterize SAC in those cells of the root contributing to the gravitropic response, followed by proof that such channels can function as gravity detectors.

Accomplishments

(1) An anion-selective SAC in tobacco cell protoplasts has been identified in membrane patches on whole protoplasts or excised from the protoplast which responds to small amounts of suction stretching the membrane (Fig. 1A). In osmoregulatory

terms, their sensitivity compares to that resulting in an increase in turgor pressure from a 1 mM increase in internal ion concentration.

(2) This SAC shows a high conductance and passes primarily anions such as chloride, but will also pass cations such as potassium and sodium in a cation:anion ratio of nearly 1:10 as determined from excised membrane patches.

(3) The average number of anion-selective SAC open increases linearly with suction.

(4) The activity of the anion-selective SAC is independent of each other. The probability that N number of SAC are open with constant applied suction follows a binomial distribution implying that there is no cooperativity among the SAC in the membrane patch (Fig. 1B).

(5) A cation-selective SAC with a smaller conductance than the anion-selective SAC has also been identified in plasma membranes of tobacco cell protoplasts (Fig. 1C).

(6) Preliminary attempts to patch root cap-derived protoplasts have proved difficult for several reasons. The primary difficulty lies in the presence of copious mucilage around the root cap which cannot be adequately removed by wiping. Detergents also harm the cells. The mucilage apparently delays and alters the enzymatic degradation of the cell wall, which in turn makes the released protoplasts less patchable. However, one can distinguish protoplasts by size and amyloplast content such that a reasonable choice can be made for those cells which are of medium size containing medium- to large- sized starch grains; these are considered to be columella derived, while larger protoplasts are thought to arise from outer cap cells and the smaller protoplasts with few or no amyloplasts are considered to be meristematic or very young cap cells. Short-lived patches have been obtained and small conductance channels have been recorded. These have not been characterized nor have SAC been sought due to the instability of the patches. Conditions for plasmolyzing cells and enzymatic treatment which release more patchable protoplasts is currently underway. Protoplasts from the root meristem and elongation zone have been obtained but have not yet been tested for patchability.

Significance of the Accomplishments

Finding #1 provides the first evidence for a possible molecular mechanism in plant membranes for the mechanotransduction of physical stimuli. In this case it is a transmembrane protein which is responsive to small changes in membrane stretch incurred by applying suction to an isolated portion of the membrane. The stretched membrane is believed to confer a change in conformation of the SAC such that selected ions will have a higher probability of passing into or out of the cell, depending on their electrochemical gradients. It is reasonable that a pressure-sensing mechanism evolved early in order to regulate cell volume. Modifications could have easily evolved with multicellular organisms and division of labor among cells such that different sets of parameters could regulate the response of

STRETCH-ACTIVATED ION CHANNELS DEMONSTRATED WITH PATCH CLAMPING

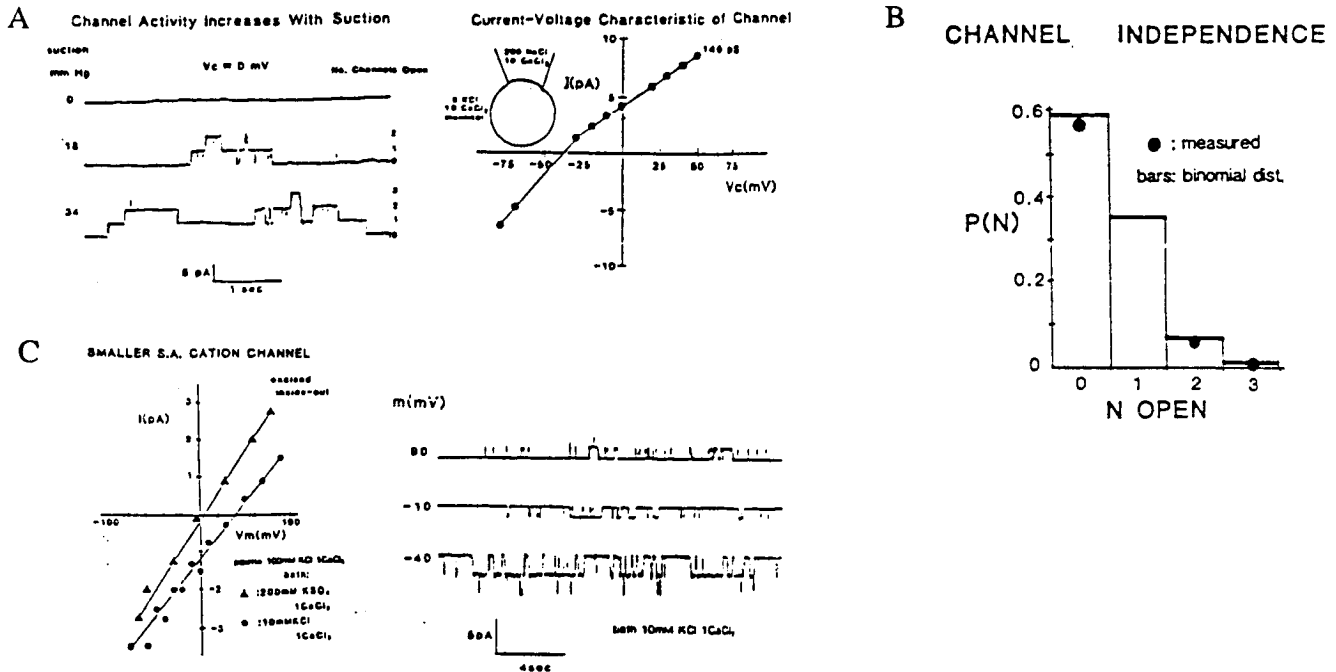


Figure 1. Stretch-activated ion channels demonstrated with the patch clamp method in plasma membranes from protoplasts of cultured tobacco stem cells. A. Anion-selective SAC responding to suction and membrane stretching, the record and a current to voltage plot of the SAC under constant suction showing the slope conductance and reversal potential indicating the anion nature of the channel. B. Anion-selective SAC independence tested by comparing the probability that N number of channels were open in a recording with constant applied suction, and the binomial distribution assuming the maximum number of channels seen (3) was the total number in the patch. C. Cation-selective SAC responding to constant suction at different clamped membrane potentials, the record and current to voltage plots. The slope conductance of this channel under the conditions used is 30-40pS.

the protein and thereby its function and sensitivity to different physical stimuli like gravity, freezing, vibration, or possibly coupled with phytochrome for the setting of the biological clock. This discovery has the potential to revolutionize our thinking about plant sensory physiology, and the patch-clamp technique is a probe to furthering our understanding of the molecular basis of these mechanisms in membranes.

Findings #2, #3, and #4 help to characterize the mechanotransductive anion channel as a probable osmoregulator. Order of magnitude calculations indicate that activation of all SA anion channels in a cell would rapidly deplete the average plant cell of all cytoplasmic ions in less than 200 msec. However, the linear response of these channels to stretch as well as the indication that these channels may not be linked to each other providing cooperative activity in the intact cell suggest that only a few channels would initially respond to increased turgor, cell swelling, with the flux of chloride or some other anion out of the cell. This flux would cause a depolarization of the cell membrane which could shift the ion preference of these channels to cations. Water would diffuse out following the loss of ions from the cell, and the cell volume would decrease, lowering the turgor pressure at the plasma membrane, thus preferentially closing the channels. That this functional interpretation for these mechanotransductive channels is mathematically feasible leads to strong belief that such channels exist in most if not all living plant cells and that looking for the functional variant for gravity detection is now justified.

Finding #5 is the first evidence for a second SAC, a cation-selective channel in plant plasmamembranes. It has a smaller conductance than the SA anion channel but its reversal potential is near that of the Nernstian potential for potassium, suggesting that its cation to anion selectivity is fairly high. The existence of this SAC lends support to the proposed osmoregulatory function of plasmalemmal SAC in cultured tobacco cells, in addition to confirming suspicions that other kinds of SAC exist in plant cells and enhancing the probability that SAC will be found in roots.

Finding #6 shows that patching of root cap-derived protoplasts is more difficult than for tobacco cells, probably because the copious mucilage of the cap both delays and alters enzymatic removal of the cell walls. However, membrane patches with low gigaohm seals are possible and small channels have been recorded. Conditions under which stable membrane patches (having an average half-life of 5-10 min) can be achieved with root cells are necessary to develop in order to examine patches for SAC or to characterize any channels found.

Publications

Edwards, K.L. and Pickard, B.G. Detection and Transduction of Physical Stimuli in Plants. In: The Cell Surface and Signal Transduction (ed. by H. Greppin, B. Millet, and E. Wagner). Berlin/Heidelberg/New York: Springer-Verlag, 1987.

Falke, L., Edwards, K.L., Misler, S., and Pickard, B.G. A Mechanotransductive Ion Channel in Patches from Cultured Tobacco Cell Plasmalemma (Abstract). Plant Physiology 80(4, Suppl.): 9, 1986.

Falke, L., Edwards, K.L., Pickard, B.G., and Misler, S. A Stretch-Activated Anion Channel in Cultured Tobacco Cells. Biophysical Journal 51: 251A, 1987.

THE ROLE OF ACID AND CALCIUM GRADIENTS IN GRAVITROPISM

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Description of Research

This research is directed toward understanding the influence of gravity on plant growth--in particular, how roots become oriented and grow in the direction of gravity (gravitropism). The detection of gravity occurs at the tip of the root while the adjustments in growth rate occur in the growing region about 0.5 cm behind the tip. During the past two years we accumulated evidence that gravity-induced redistribution of calcium within the tip of the root plays a key role in linking gravity detection to the altered growth pattern causing reorientation of the root. We also obtained evidence that the primary cause of the altered growth pattern is gravity-induced asymmetric distribution (or activity) of the growth-inhibiting hormone auxin in the growing region of the root. Possible mechanisms of action of auxin and calcium are shown in Figures 1 and 2.

During 1986, our research centered on the following aspects: (a) We know that roots can be induced to curve in a gravitropism-like manner simply by applying calcium ions to one side of the root tip. What is the cation specificity of this effect? (b) We have data indicating that the action of calcium may depend upon its ability to activate calmodulin, a widely occurring proteinaceous activator of enzymes. Does the specificity of cation action on curvature match cation action on calmodulin? (c) Considerable data indicates that the immediate cause of root curvature is asymmetric distribution within the growing zone of auxin, a hormone that inhibits root growth. Roots curve toward the side containing the highest effective level of the hormone. Is curvature induction by cations accompanied by shifts in hormone movement patterns that result in accumulation of hormone on the concave side of the curved root? (d) Roots of some cultivars of corn are not responsive to gravity when the seedling is grown in the dark. They become responsive to gravity when illuminated. We find that light stimulates the synthesis and/or activity of calmodulin in roots. But how? A recent report noted that calmodulin synthesis in plants is stimulated by vitamin D. Can vitamin D substitute for light in the induction of graviresponsiveness in roots of dark-grown seedlings? Does vitamin D play any role in the action of light? (e) Calcium movement and auxin movement appear to be closely linked. How does calcium move from cell to cell? What is the influence of auxin on this movement? Can this be studied in protoplasts isolated from root cells? (f) Is there a key tissue in the growth response of gravitropism or does the response occur uniformly throughout the growth zone?

PATTERN OF AUXIN MOVEMENT IN ROOTS

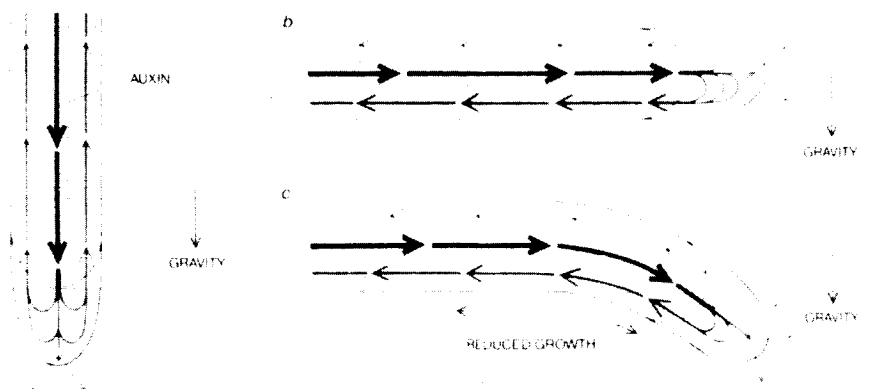


Figure 1. Diagram representing the pattern of flow of auxin (a hormone that inhibits root growth) in vertical and gravistimulated roots. In vertically oriented roots (left), the auxin flows toward the root tip through the central part of the root. Once the auxin enters the root tip it is redistributed so that much of it is sent back toward the growing portion of the root (behind the tip) in a symmetrical pattern through the cells around the root periphery. The action of auxin on cells around the root periphery is thought to control root growth, while auxin in the root's center is believed to be transported without influencing growth.

When the root is oriented horizontally (upper figure), auxin moving into the root tip is thought to be rerouted primarily downward and then back into the growing cells along the lower side of the root. The accumulation of auxin in cells along the lower side of the growing region slows growth there, causing the root to bend downward (lower figure).

PROPOSED MODEL OF SENSING AND TRANSDUCTION STAGES OF GRAVITROPISM

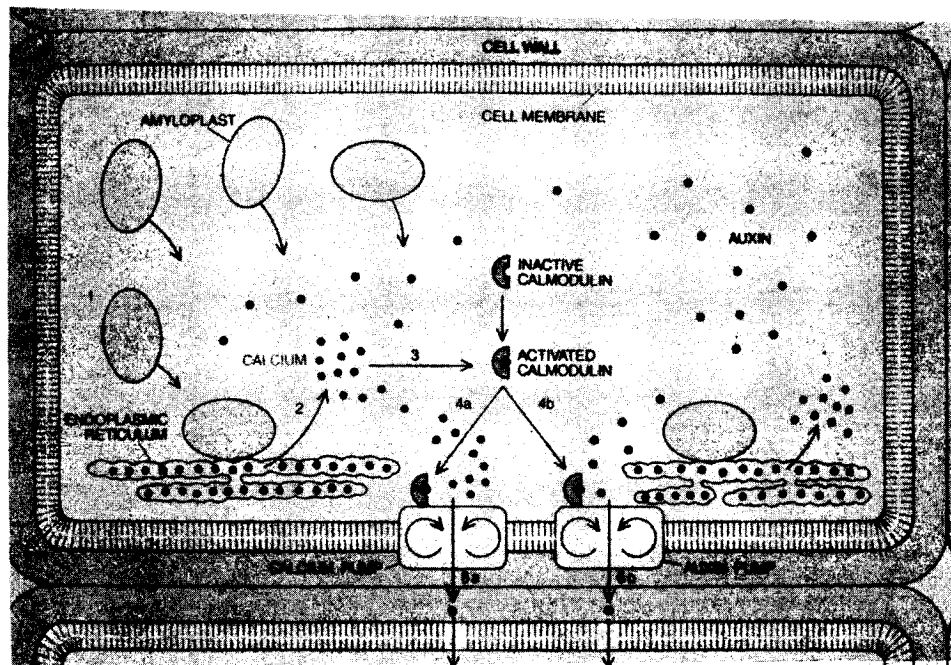


Figure 2. Model explaining how the sedimentation of amyloplasts to the bottom of gravity-sensing cells in the root tip might lead to the downward movement of both calcium and auxin toward the lowermost side of the root. The large oblong structures represent amyloplasts, dense bodies that sediment to the lower edge of the cell due to gravity. In this model, the falling amyloplasts contact a membrane system (endoplasmic reticulum) on the lower edge of the cell causing calcium ions to be released into the cytoplasm. Calcium is thought to bind to and activate a special protein called calmodulin. According to this model, when calmodulin is activated, it in turn activates two membrane-localized pumping systems, a calcium pump and an auxin pump. These two pumps move auxin and calcium toward the bottom of the root tip and from there the growth-inhibiting auxin is sent back (via a calcium-requiring mechanism) along the lower side of the root where it inhibits growth, causing the root to bend downward.

Accomplishments

(1) Induction of root curvature by unilateral application of cations is not restricted to calcium. Roots curve toward calcium and higher concentrations of copper, but away from aluminum, barium, cadmium, and low concentrations of copper (Figure 3).

(2) Calcium, which induces root curvature toward the source, activates calmodulin, while aluminum, which induces curvature away from the source, inhibits calmodulin.

(3) Calcium applied to the root tip promotes auxin movement from the tip back into the growing zone where curvature occurs. Aluminum inhibits the movement of auxin from the root tip back toward the growing zone.

(4) Some forms of vitamin D are capable of inducing gravitropic responsiveness of roots of dark-grown seedlings of corn that normally require illumination in order to respond to gravity.

(5) Viable protoplasts can be isolated from roots and they exhibit reproducible calcium uptake and efflux kinetics. Auxin stimulates the release of calcium from root protoplasts.

(6) In a more complete study of the tissues involved in root gravitropism, we found the outer cell layers to be especially significant. Roots lacking epidermal cells grow but are not able to respond to gravity.

Significance of the Accomplishments

Finding #1: Cations other than calcium can induce root curvature. Our model has focused on gravity-induced calcium redistribution as a key element in transduction of the gravitropic stimulus. The model suggests that the action of calcium in turn depends upon its ability to activate calmodulin. These results show that cations other than calcium are effective. This causes us to reevaluate the model, taking into account modes of action not necessarily mediated by calmodulin.

Finding #2: Calcium and aluminum have opposite effects both on curvature and calmodulin activity. This, plus the fact that the other cations tested also influence calmodulin activity, indicates that the model of calmodulin involvement in transduction or curvature is still viable. The fact that these cations have qualitatively different effects on curvature which parallel their effects on calmodulin is consistent with our model.

Finding #3: Calcium promotes auxin movement toward the growing zone while aluminum inhibits this movement. This is particularly significant because it allows an explanation of the effects of these cations on root curvature which fits both the predictions of our model and the evidence that the distribution of the hormone auxin ultimately controls root orientation. For example, aluminum applied to one side of the root tip reduces movement of auxin back into the growing region along that side and the root

curves away from the site of application of aluminum. We feel that these effects are causally related and that they reflect the basic mechanism behind gravitropic curvature.

Finding #4: Vitamin D can induce graviresponsiveness in roots of dark-grown corn seedlings which would otherwise require light for gravitropic sensitivity. Since this experiment "works" with some sources of vitamin D but not others, we are skeptical of its physiological relevance. However, if it can be shown to be reproducible and if the active component can be identified, this observation will be of great significance since we do not know how light induces gravitropic sensitivity. The fact that vitamin D is reported to induce de novo calmodulin biosynthesis in plants increases our interest, since photoinduction of gravitropic competency in these roots is preceded by enhanced calmodulin biosynthesis.

Finding #5: Auxin stimulates the release of calcium from isolated root protoplasts. We do not know how auxin movement and calcium movement are related in roots. Our model holds that elevated levels of calcium enhance auxin loading into the transport stream for this hormone. The experiments with protoplasts support this idea since one would expect enhanced calcium efflux from protoplasts in the presence of auxin if the entry of auxin is linked to calcium counter movement or to transient opening of a calcium gate.

Finding #6: Removing epidermal cells prevents the response of roots to gravity. This confirms earlier preliminary studies. These findings indicate that the epidermis may be the key tissue controlling growth in roots. If so, these cells may be the site at which the major growth adjustments are made in response to gravistimulation. This could be very important in characterizing the gravity response since it will direct the search for causes of differential growth to the responding cells and avoid potentially misleading clues from cell types less directly involved.

Publications

Evans, M.L. Role of Calcium in Gravity Perception of Plant Roots (Abstract). In: Twentieth-Sixth Plenary Meeting of the Committee on Space Research, Abstracts. Paris: COSPAR, p. 330, 1986.

Evans, M.L., Moore, R., and Hasenstein, K.-H. How Roots Respond to Gravity. Scientific American 254: 112-119, 1986.

Hasenstein, K.-H. and Evans, M.L. Calcium Dependency of Ethylene Production in Segments of Primary Root of Zea mays. Physiologia Plantarum 67: 570-575, 1986.

ROOT CURVATURE DUE TO APPLICATION OF CATIONS



Figure 3. An example of cation-induced curvature in roots of corn seedlings. In each case a small block of agar containing aluminum (Al^{+++}) was applied to the right side of a root tip of a seedling growing in the normal vertical orientation. A plain agar block was applied to the opposite side. Within 90 minutes the roots curved strongly away from the Al^{+++} . Certain other cations are also capable of inducing curvature when applied in this way. Research is being done to determine whether gravity stimulation causes natural gradients of cations to develop.

Hasenstein, K.-H. and Evans, M.L. The Calcium Dependence of Rapid Auxin Action in Maize Roots. Plant Physiology 81: 439-443, 1986.

Hasenstein, K.-H. and Evans, M.L. Uptake and Release of Calcium and Auxin by Maize Root Protoplasts (Abstract). Plant Physiology 80(4, Suppl.): 60, 1986.

McFadden, J. and Evans, M.L. The Effect of Calcium Entry Blockers on the Sensitivity of Maize Roots to Auxin (Abstract). Plant Physiology 80(4, Suppl.): 119, 1986.

Nelson, A. and Evans, M.L. Analysis of Growth Patterns During Gravitropic Curvature in Roots of Zea mays using a Computer-based Video Digitizer. Journal of Plant Growth Regulation 5:73-83, 1986.

Nelson, A. and Evans, M.L. Analysis of Growth Patterns During Gravitropic Curvature in Roots of Zea mays using a Computer-based Video Digitizer (Abstract). Plant Physiology 80(4, Suppl.): 7, 1986.

Stinemetz, C., McFadden, J., and Evans, M.L. The Light-dependency of Ethylene Sensitivity and Auxin-induced Ethylene Synthesis in Roots of Maize (Abstract). Plant Physiology 80: (4, Suppl.) 119, 1986.

PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES ASSOCIATED WITH GRAVITROPISM IN ROOTS OF MAIZE

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Description of Research

On Earth roots typically respond to gravity by growing downward. Our research focuses on the physiological and biochemical steps involved with transducing the gravity stimulus in roots of maize.

We know that gravity is perceived in a specialized region of the root, the root cap. As a result of gravity perception it is hypothesized that a signal or message is produced in the cap and that this message in some way is involved with downward curving of roots. In the past we have investigated the chemical nature of the hypothesized message. More recently, and during the past year, we have studied the physiological and biochemical steps which need to occur in the root cap in order for the hypothesized message to be produced. The results from that effort form the body of this summary.

For our work we have used a mutant of corn in which gravitropic bending occurs only if the roots are exposed to light. If roots are grown in darkness they do not grow downward, but rather grow parallel to the surface on which they are supported. Thus, it is hypothesized that in these light-requiring mutants, illumination triggers processes requisite for normal gravitropic root bending. Using dark-grown roots we have investigated the occurrence and level of specific biochemical and physiological processes within the cap. We then illuminate roots and measure the levels of these same processes or events. In this way we hope to understand how the gravity stimulus is processed by the root cap.

Accomplishments

The major findings from these studies are:

(1) Red light is the most effective wave length of light for inducing gravitropic bending.

(2) The amount of red light needed for this response is exceedingly low, approximately 10^{-7} moles m^{-2} , and this response is not reversible with far red light.

(3) Illumination stimulates both protein and mRNA (messenger RNA) synthesis in the root cap. New proteins are detected within 30-60 min following illumination and new mRNA within 15 min of illumination.

(4) Having shown that illumination leads to a general increase in proteins and mRNA in root caps, we also have identified and measured the levels of specific proteins and their messages. Of these proteins, we have concentrated on one in

particular, calmodulin. Within 30 minutes of illumination there is a 4-fold increase in the level of calmodulin in root caps. The mRNA for calmodulin rises rapidly following illumination and precedes the light-stimulated increase in protein.

Significance of the Accomplishments

We are interested in the mechanism for transducing the gravity stimulus in roots. A perfect control would be to place roots in a totally gravity-free environment and then determine the base levels of various processes. Roots would then be returned to a plus-gravity environment and we would determine what new processes have been initiated (or retarded). However, for the present, a perfect zero-gravity control is not available. For our control, therefore, we have used roots which respond only incompletely to gravity when these roots are maintained in the dark. Illumination causes the roots to grow downward. Our working hypothesis is that light triggers steps necessary for processing the gravity stimulus. Using this system, we have investigated the processes in the root cap affected by light.

First, we have shown that the response is mediated by red light of an exceedingly low dosage. Such low levels of light can be recorded at quite deep levels in the soil. This suggests that for many plant roots light may be a normal component of the gravity processing mechanism. Because this response is mediated by red light, the candidate photoreceptor is the pigment phytochrome. Indeed, we have shown that in the maize cultivar we use, root gravitropism is mediated by phytochrome. Moreover, because of the low level of light required, this response is characterized as a very low fluence phytochrome response.

Light also stimulates both protein and mRNA synthesis within the root cap. Some time ago we showed that protein synthesis within the cap was necessary in order for light to induce gravitropism in roots. During the past year we have identified several of these proteins. In particular, we have shown that the protein calmodulin increases in root caps when illuminated, and that the kinetics of this increase precede gravitropic root bending. We consider this a significant observation. The protein calmodulin is believed to regulate plant development by combining with calcium which in turn affects other developmental processes. Our data suggest that the now known effects of calcium on root gravitropism may in part be mediated by an increase in the level of the calcium-binding protein, calmodulin. We also have obtained information on the likely mechanism by which light regulates calmodulin levels. Our preliminary data suggest that this regulation in part may be at the level of the mRNA for this and other proteins. We have shown rapid effects of light on mRNA for a number of light-stimulated proteins. Thus, a possible interaction between light and gravity may be at the level of messenger RNA.

Publications

Feldman, L.J. and Briggs, W.R. Light-regulated Gravitropism in Seedling Roots of Maize. Plant Physiology 83: 241-243, 1987.

Feldman, L.J. and Sun, P.S. Effects of Norflurazon, an Inhibitor of Carotenogenesis, on Abscissic Acid and Xanthoxin in the Caps of Gravistimulated Maize Roots. Physiologia Plantarum 67: 472-476, 1986.

CHARACTERIZATION OF NEUROSPORA CIRCADIAN RHYTHMS IN SPACE

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Description of Research

The ultimate long-range goal of our research is to determine whether circadian rhythms, thought to be endogenously derived, can persist normally in the absence of all known geophysical and environmental cues. A rhythm is thought to be circadian if it can persist in constant conditions, free-run with an endogenously derived period of about 24 hr in these constant conditions, and entrain to 24 hr environmental time cues. It has been proposed by some that the free-running rhythms we observe, in conditions which we consider constant (i.e., constant light, temperature, and humidity), are not free-running rhythms in constant conditions at all; rather, rhythmic phenomenon in known and unknown geophysical cycles induce rhythmicity in the organisms. It is their hypothesis that there are no circadian rhythms driven by an "internal clock," only passive oscillations responding to rhythmic environmental influences. By observing the rhythmicity of an organism in an environment removed from the geophysical rhythms of Earth, we can determine whether these rhythms are endogenous by their ability to persist without these purported exogenous time cues.

The filamentous fungus, Neurospora crassa, displays rhythmic growth patterns in certain specific conditions. This rhythmic growth consists of an alternating low growing surface mycelium and a surface mycelium with aerial hyphae which pinch off to form conidia (asexual spore formation). The mycelium that contains the aerial hyphae is clearly seen as a band when grown on media in petri dishes or long cylindrical tubes, known as race tubes (Figure 1). In constant conditions the cycle of banding is repeated approximately once every 22 hr (i.e., the rhythm is in the circadian range).

To determine if this circadian rhythm of conidiation is endogenously derived or is driven by some geophysical time cue, an experiment was conducted on Shuttle flight STS-9 where race tubes inoculated with growing Neurospora were exposed to the microgravity environment of space. The results demonstrated that the rhythm can persist in space. However, there were several minor alterations noted. There was an increase in the period of the oscillation and the variability of the growth rate. Furthermore, the rhythm of the conidiation possessed a diminished amplitude and eventually damped out in 25% of the flight tubes. However, on day 7 of the flight, the tubes were exposed to light while their growth fronts were marked. It appears that some

NEUROSPORA ORIENTATION STUDIES

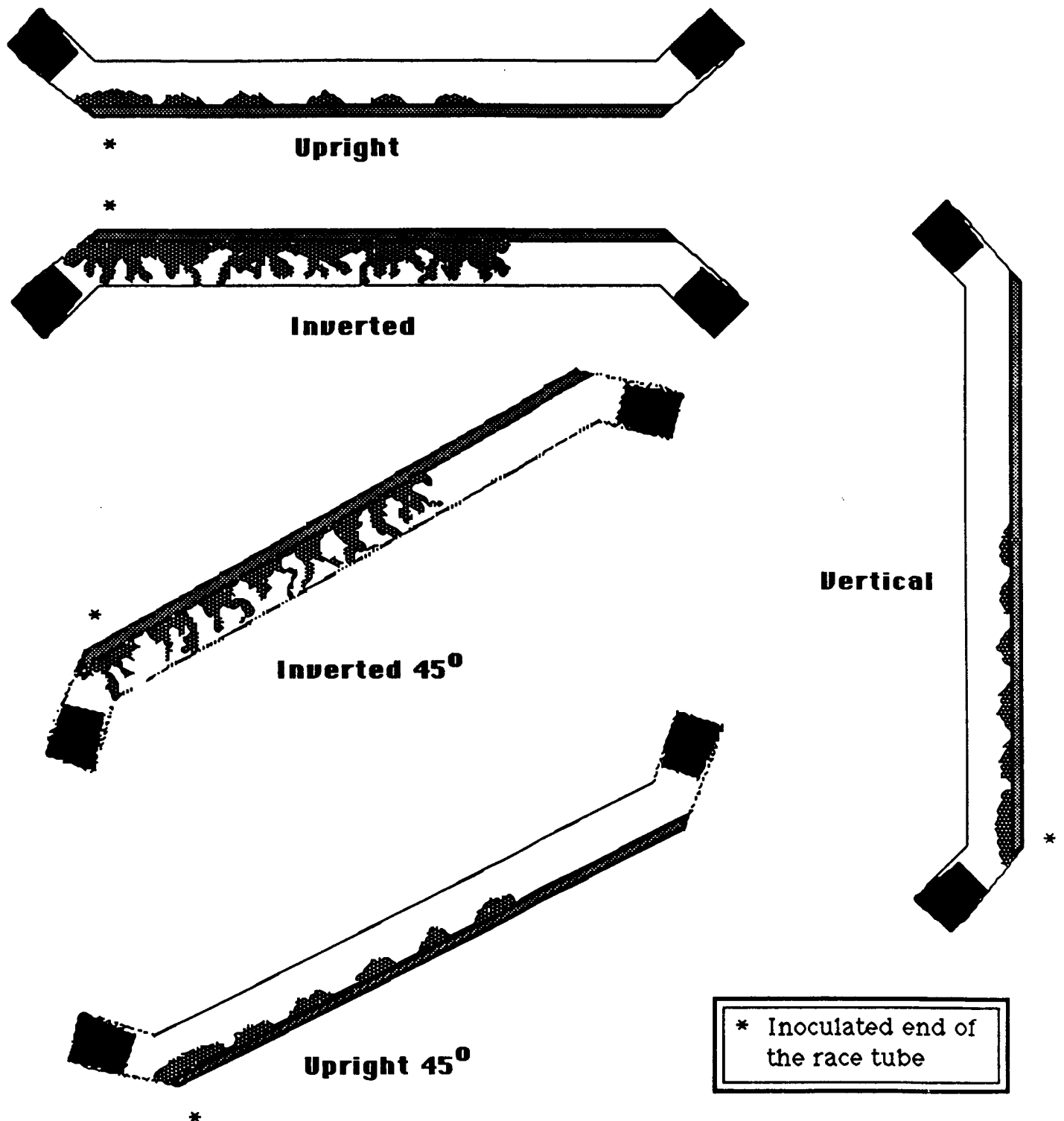


Figure 1. Rhythmic growth patterns of *Neurospora crassa* in various 1 g orientations. The horizontal upright tube was the control. The horizontal inverted tube showed decreased amplitude and rhythmic damping, while the inverted 45° tube showed similar results but more pronounced. The vertical and upright 45° tubes showed no changes.

aspect of this marking process reinstated a robust rhythm in all the tubes which continued throughout the remainder of the flight. These results lead us to question of why the rhythm, before the marking procedure, had been so variable and even damped out in some cases.

Much of the past year has been devoted to the materials, hardware, and procedures in preparation for our middeck experiment BGE NS402, which was scheduled to fly on EOM 1/2 in 1986. Due to the substantial postponements in the flight schedule, we shifted our major emphasis from flight preparation to investigating those aspects of the question that can be examined utilizing ground-based research. This include experiments on the effects of: (a) clinostat rotation simulating 0 g (b) different orientations of the 1 g gravity vector, (c) chronic and acute exposure to hypergravity (3 g), and (d) genetic strain, the rhythmic conidiation and growth of Neurospora.

Experimental cultures are grown by inoculating one end of a "race" tube containing medium. Cultures are grown in constant bright light for 24 to 48 hr, then the growth front is marked on the glass tube and the tubes are placed in constant darkness. The growth fronts are marked at regular intervals during and at the end of an experiment under a red "safe" light which has been shown not to affect the free-running conidiation rhythms. Since growth is linear at constant ambient temperatures, the time of occurrence of each conidiation band can be determined from the growth front marks.

Accomplishments

(1) Design Changes of NS402: Increase of sample size; subdivision of the package into three sections; addition of a second strain of Neurospora (CSP); addition of gas sampling syringes; earlier marking of the tubes in flight.

(2) Clinostat Studies: A clinostat rotates the race tubes about their axes, thus distributing the gravity vector equally in all directions, simulating 0 g. The rhythm damping and the decreased amplitude seen in orbit on STS-9 was not simulated by the clinostat at any of the circadian times; the growth rate was significantly slower on the clinostat.

(3) Orientation Studies: Several different orientations of the 1 g gravity vector were studied: (a) horizontal and upright (control); (b) horizontal and upside down; (c) vertical; (d) 45° angle with the media upside down; (e) 45° angle with the media upright (Figure 1). Neurospora in group b displayed a decreased amplitude and rhythm damping (one tube became arrhythmic) and the period was significantly increased. Neurospora in group d showed similar but more attenuated results. Neurospora in groups c and e displayed no damping or changes in amplitude. Neurospora growing vertically showed a decreased growth rate.

(4) Hypergravity Studies: Chronic (7 day) and acute (10 min) exposure to a 3 g load was performed; CSP and BND strains were used; the onset of the 3 g exposures occurs at circadian times (CT) 3, 9, 15, & 21. Two groups of the acute exposure group were exposed to light shortly after being removed from the centrifuges. The major findings were:

a) chronic exposure of Neurospora to a 3 g force has no damping effect

b) acute 10-min (as in lift-off) exposure to this hypergravity force causes significant damping of the circadian rhythm of conidiation

c) a brief light pulse given 36 hr after the acute exposure eliminates any effect of the acute 3 g exposure on damping

d) BND is more susceptible to the hypergravity perturbation than CSP

e) the average free-running period increases with chronic hypergravity

f) the major effects observed in the STS-9 experiment were simulated by hypergravity, including the ability of a light pulse to correct the aberrations.

Significance of the Accomplishments

The increased number of experimental samples will not only aid in our statistical analysis, but has also allowed us to divest our samples into a greater number of paradigms; thus resulting in the equivalent of several flight experiments in one. The CSP strain grows at a somewhat faster rate and "bands" with an increased resolution and clarity when compared to BND. This second fact becomes very significant in light of the results of STS-9, where a significant number of samples appeared to become arrhythmic (i.e., no clear banding) prior to the marking procedure. Gas sampling syringes were added to the package to determine if, in the unique conditions of space, the gaseous environment within the race tube had a significantly higher concentration of CO₂ at the growth front, (CO₂ is known to inhibit rhythmicity and therefore banding). Marking the tubes earlier in the flight will hypothetically reinstate rhythmicity soon after the hypergravity pulse disrupts it and thus determine if the microgravity environment of Earth orbit can support the normal rhythm of conidiation. This procedure will allow sufficient time for the rhythm to damp after the marking procedure, if indeed the damping is due to being in space and not due to the hypergravity of launch.

Ground-based experiments were conducted in order to develop a ground-based paradigm that could simulate the effects of microgravity on the Neurospora rhythm of conidiation (i.e., increased period length, decreased rhythm amplitude, rhythm damping, and increased variability in growth rate). While the clinostat studies failed to simulate the effects of microgravity on the conidiation rhythm, the orientation studies demonstrate that creating a gravity vector 180° from normal is a relatively good model to simulate the spaceflight results (Ferraro, et al.,

1986).

The results obtained in the hypergravity study are even more promising and support our hypothesis that the hypergravity of launch caused the damping, arrhythmicity and the increase in period length of the Neurospora circadian rhythm of conidiation, rather than the absence of geophysical cues found in space. Furthermore, it is also quite evident that the BND strain is more susceptible to the hypergravity perturbation than the CSP strain. Thus, flight results obtained with CSP should be cleaner. Similar to what is seen during spaceflight, the average free-running period increases with chronic hypergravity. Furthermore, the period of each individual cycle continues to increase throughout the exposure period. At this point in time, it appears that the hypergravity of launch can severely affect the physiological timekeeping system and may account for most of the aberrant effects observed on STS-9.

MECHANISMS OF GRAVIPERCEPTION AND TROPISTIC RESPONSE IN PEA SEEDLINGS

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Description of Research

The ultimate objective of our research is the complete understanding of the upward tropistic curvature of plant stems in response to the Earth's gravitational field, as well as of the physical and chemical mechanisms underlying the response. Throughout our studies, we have used etiolated pea epicotyls (cv. Alaska), either as entire 3rd internode or as 5-7 mm segments taken from the apical part of 3rd internode. In most cases, ^3H -IAA and $^{45}\text{Ca}^{2+}$ have been used to follow indole-3-acetic acid and calcium movements into plant tissues and protoplasts, but in some cases spectrophotofluorimetric techniques have been used to monitor calcium movement as well.

From previous research, we know that curvature is associated with preferential movement of auxin toward the lower part of gravistimulated stems. The resulting accumulation of auxin on the lower side of the stem is thought to induce there an accelerated growth and thus a curvature away from the Earth's center of gravity. Several recent publications, principally concerned with roots, have stressed the importance of a concomitant movement of calcium ions as regulators of IAA movement and action in the early phase of the graviresponse. Thus, an extension of the last year's investigations on the relation between IAA and Ca^{2+} in the stem's response to gravity appeared desirable.

This year's investigations concern the effects of calcium ions and of factors affecting Ca^{2+} uptake into cells on IAA uptake into etiolated pea epicotyl segments, and on IAA transport, redistribution, and tropistic curvature in entire 3rd internodes of such pea epicotyls. We were able to link this work with our previous research on polyamines, which affect Ca^{2+} uptake and movement in pea stems, as well as Ca^{2+} movement into and out of pea leaf protoplasts. Protoplasts were used to study calcium movement through membranes in cells deprived of cell walls, since we found that over 80% of pea stem calcium is located in the walls.

Accomplishments

- (1) Ca^{2+} strongly increases IAA uptake into pea epicotyl segments. It slightly inhibits growth, but does not increase IAA redistribution or gravicurvature.
- (2) La^{3+} can substitute for Ca^{2+} as a stimulator of IAA

uptake and, since it has been reported not to penetrate plant cells, we conclude that its action, and the similar action of Ca^{2+} , are exerted from the outside of the cell.

(3) Spermidine (Spd, a triamine) has a complex action on IAA uptake, stimulating it in the first hour and then strongly inhibiting it. The inhibition of IAA uptake, IAA redistribution and gravicurvature by Spd seems to be related to its ability to affect Ca^{2+} uptake and titer. Spd in fact inhibits Ca^{2+} uptake into and increases Ca^{2+} release from pea epicotyl segments.

(4) EGTA has an effect similar to that of Spd, but considerably milder. Only a long treatment with 1 mM EGTA will produce a clear inhibition of IAA uptake, IAA redistribution, and graviresponse.

(5) Chlorpromazine, an inhibitor of calmodulin, effectively inhibits IAA uptake, IAA redistribution, and graviresponse. This confirms previous work of Roux et al. and supports the possibility that Ca^{2+} and calmodulin could play a central role in early phases of the graviresponse.

(6) TIBA, an inhibitor of IAA transport, strongly inhibits IAA uptake, IAA redistribution, and the graviresponse. This action supports the importance of auxin in the response to gravity by plant stems.

(7) Chlorpromazine and TIBA do not affect Ca^{2+} uptake over an initial 2-4-hr period. This is probably due to the fact that most of Ca^{2+} is taken up into free space, e.g. a compartment insensitive to the above substances.

(8) Ca^{2+} increases the length of the zone of pea epicotyl responding to gravity, whereas spermidine, chlorpromazine and TIBA reduce it. The effects seem correlated with auxin transport.

(9) Spd inhibits Ca^{2+} uptake into and release from pea leaf protoplasts. This shows that its action does not concern only Ca^{2+} in the free space, but also penetration through plasmalemma.

Significance of the Accomplishments

These results suggest that Ca^{2+} must be present in millimolar concentration in cell walls and other apoplastic free space to insure normal IAA movement, IAA redistribution, and gravicurvature. The effect of Ca^{2+} seems to be exerted from outside the cells, since La^{3+} , known as a nonpermeant substance, can substitute for it.

Spermidine and other polyamines, known to occur in cell walls, can affect Ca^{2+} titer and movement, and thus various aspects of the gravitropic response. Through its control of Ca^{2+} permeation into the cell, Spd may also control Ca^{2+} -regulated cellular events.

The fundamental role of IAA in the graviresponse is reinforced, since TIBA, spermidine, and EGTA, all of which inhibit IAA movement directly or indirectly, also affect the response to gravity of pea epicotyls.

Ca^{2+} also appears to be strictly implicated in the graviresponse, since the removal of calcium from free space by displacement (Spd) or chelation (EGTA) or the inhibition of calmodulin by chlorpromazine reduces the graviresponse.

Publications

Migliaccio, F. and Galston, A.W. A Reinterpretation of Ca^{2+} Movement During Gravitropism (Abstract). Plant Physiology 80 (4, Suppl.): 8, 1986.

MECHANISM OF GRAVITY RESPONSES IN CEREAL GRASS SHOOTS

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Description of Research

Our long-range goal in this study is to unravel the basic mechanism underlying the negative gravitropic curvature response in gravistimulated cereal grass pulvini. It is thus concerned with how gravity is perceived in this system, how and when the gravity signal gets transduced, and as a consequence of these two steps, how the unequal growth response occurs. In this effort, we are developing a rather unique model that explains the cascade of events underlying this upward bending response in gravistimulated cereal grass shoots.

Our primary objectives include the following: (a) determining what role(s) the starch statoliths play in the graviperception mechanism; (b) establishing when and how hormone asymmetries develop in the pulvinus in relation to the time when upward bending first commences in pulvini of prostrated shoots; and (c) determining the effects of gravistimulation on cell wall composition, levels of starch, and synthesis of growth-related proteins so as to shed light on the metabolic basis for the response mechanism.

Specifically, our gravity perception studies are concerned with these questions: (a) Are starch-containing plastids in the pulvinus the true gravireceptor organelles in this system? (b) If so, what are their functions as gravireceptors? (c) What are the fine kinetics for time of initiation and rate of curvature in upward bending pulvini, and do these parameters change significantly in pulvini which are regravistimulated by rotating them 180° several times in succession? (d) Do cells in the upper portions of graviresponding pulvini get compressed, and if so, does the upper side actually become "accoridianated" (shrunk) during upward bending? (e) How many times can a pulvinus respond to repeated gravistimulation treatments; that is, how many times can a pulvinus reverse curvature after 180° "flip-flop" treatments?

Our analysis of gravity transduction focuses on the idea that hormones such as IAA (indole-3-acetic acid) and GAS (gibberellins) become asymmetrically distributed in response to gravistimulation of the pulvinus. Since there is no evidence for basipetal transport of either type of hormone in cereal grass pulvini, we are testing the idea that the asymmetry arises as a consequence of differential synthesis and/or release of free

hormones from their respective conjugates. The use of the 'lazy' mutant of corn (Zea mays) is proving to be of enormous help in these hormone studies.

In connection with our studies on the biochemical/physiological basis for asymmetric growth in gravistimulated pulvini, we are currently focusing on changes in key cell wall constituents (wall proteins, cellulose, neutral sugars, reducing ends released by beta-glucanase), starch, and cytosolic proteins elicited by gravistimulation. Most recently, we have found that gravistimulation causes major changes in: (a) patterns of protein synthesis in upper and lower halves of the pulvinus, (b) starch composition of the pulvinus, and (c) levels of glucose in the respective halves. The kinetics for these changes are currently being examined in relation to the time of initiation of upward bending.

Accomplishments

(1) Graviperception in Cereal Grass Pulvini

(a) To test the idea that starch statoliths are the graviperceptive organelles in cereal grass pulvini, we placed 45-day-old 'Larker' barley plants in the dark at room temperature for five days to deplete the pulvini of all starch. When such starch-depleted pulvini were gravistimulated, they showed no upward bending response over a 24-hr period. However, when these pulvini were fed 0.1M sucrose during a 24-hr gravistimulation period, they reformed starch grains in the statocytes and also showed an upward bending response of 39°. These results clearly support the idea that starch-containing statoliths in statocyte cells of cereal grass pulvini are indeed the graviperceptive organelles in this system.

(b) Kinetic analyses on the time-course and rate of upward bending in gravistimulated cereal grass pulvini were performed with an angular recording transducer. In 'Victory' oat shoots, the lag period before upward curvature is initiated is 58 min on average, and the rate of curvature during linear phase of bending is 1.5° per minute (over approximately a 24-hr period), after which time the rate gradually decelerates over the next 48 hrs to zero. The initial response to gravistimulation is downward bending ("wrong-way curvature"); this is then followed by a gradual upward movement of the shoot back to the horizontal, and by 58 min, it starts to show a positive gravitropic response.

(c) Using scanning electron microscopy, we have demonstrated that during the course of upward bending in a gravistimulated pulvinus, parenchyma cells just inside the epidermis on the upper side become visibly compressed, whereas no such compression occurs in elongating cells in comparable positions on the lower side. The epidermal cells on the upper side do not show this compression, nor do they become corrugated as a result of gravistimulation of the pulvinus.

(d) To determine how many times a single pulvinus can respond to repeated gravistimulation treatments, we used both intact plants and excised pulvinus-containing stem segments and a

protocol involving 180° "flip-flop" rotations of the horizontally placed pulvinus each time it had responded to gravity. A given pulvinus can respond by reverse curvature as many as six times after repeated 180° rotation treatments. The lag is always the same (about 58 min), but after each new reverse curvature response, the rate of upward bending increases significantly from the one previous. As a consequence of such responses, the pulvinus elongates to almost 10 times its original length.

(2) Transduction in Cereal Grass Pulvini

(a) Using the double isotope dilution method of J. Cohen and R. Bandurski, we have shown that a 1:2.5 free IAA top/bottom asymmetry develops in gravistimulated oat leaf-sheath pulvini within three hours and persists after 6, 12, and 24 hr of gravistimulation. Interestingly, in upright, control leaf-sheath pulvini, IAA conjugates predominate over free IAA in a ratio of 3:1. Current efforts are directed at checking the free IAA and IAA conjugates in pulvini that have been gravistimulated for much shorter time periods (1, 3, 5, 15, 30 and 60 min).

(b) Studies on the native gibberellins in normal and lazy corn show that in gravistimulated leaf-sheath and internodal pulvini, free GAs accumulate in the lower halves and GA conjugates in the upper halves in normal corn, but in the lazy corn mutant, no such asymmetry in free GAs and GA conjugates develops in the prostrate shoots of these plants. We are currently determining when the free GA and GA conjugate asymmetry first develops in gravistimulated shoots of normal corn plants.

(3) Gravity Response Mechanism

(a) Gravistimulation results in significant changes in patterns of protein synthesis in cereal grass pulvini: in barley, there is enhanced synthesis of at least four buffer-soluble proteins in the bottom halves of the pulvinus as a result of gravistimulation. Labelling with S_{35} methionine and fluorography show the "lighting-up" occurs on SDS/PAGE gels for 44, 48, 59, and 79 Kd proteins in the bottom halves after 12 and 24 hr of gravistimulation. For buffer-insoluble proteins, we see enhanced synthesis of a 47 Kd protein in the top halves of pulvini within 6 hr after gravistimulation, and likewise, of 25, 32, and 47 Kd proteins in the bottom halves. Synthesis of these proteins is most strongly expressed after 24 and 48 hr of gravistimulation.

(b) Studies on the role of calcium in the asymmetric growth response in oat leaf-sheath pulvini indicate that lateral application of $10\ \mu\text{M}$ IAA generates an angle of bending in vertical segments, over 24 hr, that is comparable to that in gravistimulated horizontal segments; and that application of IAA with either verapamil or LaCl_3 (calcium channel blockers) significantly inhibits pulvinus growth and segment bending, as compared with IAA alone. Treatment with IAA plus EGTA (a calcium chelator) is comparable in effect to IAA alone.

(c) Leaf-sheath pulvini of barley, when placed in sterile culture on a 2-MS medium (2 mg/l 2,4-D in Murashige and Skoog medium) develop prolific callus tissue within 5 to 6 days. We shall be using this callus tissue to make protoplasts to test the idea that membrane channels (e.g., for IAA, GAs, Ca^{2+} , and protons) must be open for a graviresponse to occur. We shall be

using the patch-clamp techniques (see K. Edwards' and B. Pickard's summaries) to test this idea.

(d) Gravistimulation has a marked effect on several metabolites, including cell wall constituents: in oat leaf-sheath pulvinus tissue, levels of pulvinus starch drop significantly, especially in the bottom halves, and at the same time, levels of D-glucose (mole percent of sugars in the hemicellulose fraction) increase almost 40% in the lower halves of such pulvini, with no change occurring in the top halves. Gravistimulation has no effect on levels of uronic acids (pectins) of the cell walls nor on amounts of neutral sugars other than glucose (fructose, rhamnose, arabinose, xylose, mannose, and galactose) in the hemicellulose wall fraction. However, it does result in a greater than 90% increase in nanomoles of reducing ends released by beta-glucanase per mg of wall in lower halves of pulvinus tissue. This could explain the significant increase in D-glucose in these halves as a result of gravistimulation. All these results are based on analyses of 50 pulvini gravistimulated for 48 hr in comparison with upright control pulvini. The mean angle of upward bending was 20°.

Significance of the Accomplishments

Finding #1. Starch statoliths which occur in abundance in statocyte cells located just inside each vascular bundle in the pulvinus can now be said with assurance to function as the graviperception organelles that trigger the upward bending response in gravistimulated cereal grass shoots. We propose three possible functions: (a) they may serve as a source of substrate for wall biosynthesis; (b) they may act as pressure probes to open hormone and/or ion channels in the plasma membrane; and (c) they may act as information carriers by bringing deconjugating enzymes to the plasmalemma so that free hormones such as IAA and GAs are released from their conjugates. Each of these ideas is testable and will be examined.

Finding #2. Two types of hormones now appear to be essential to the transduction process in cereal grass pulvini as they bend upward in response to gravistimulation: auxin (IAA) and gibberellins (GAs). Gravistimulation of the pulvinus results in the accumulation of active, free GAs in the lower halves and inactive GA conjugates in the upper halves; likewise, free IAA also accumulates in significant amounts in the lower halves but not in the upper halves. How and when these asymmetries in GAs and IAA are established are now the primary questions we are attempting to answer. One clue as to how the IAA asymmetry is established is found in the upright, non-gravistimulated pulvinus. Here, we find that IAA occurs primarily in its conjugated form (mostly as amide-linked IAA). Is it not possible that at least part of the IAA asymmetry in the gravistimulated pulvinus arises as a result of enhanced release of free IAA from its conjugate in the lower halves of the pulvinus? This idea is currently being tested in addition to the possibility that part of the asymmetry arises as a result of differential synthesis of IAA in the upward bending pulvinus. We are also now looking at

the very early kinetics for the establishment of asymmetries in free GAs and their conjugates and free IAA and its conjugates in the upward bending pulvinus, to establish when the asymmetries are first seen in relation to the time when upward bending is initiated (about 58 min on average for oat pulvini).

Finding #3. We now know that gravistimulation triggers important changes in cellular metabolites that play key roles in the upward bending response mechanism. The hydrolysis of starch to D-glucose and the release of D-glucose and D-fructose from sucrose because of enhanced invertase activity in the lower halves provides a ready pool of substrate for the enhanced wall synthesis that accompanies the enhanced cell elongation in this portion of the pulvinus. The enhanced release of D-glucose from hemicellulose in the cell wall by beta-glucanase as upward bending is occurring could be a key factor in the wall-loosening process that leads to cell elongation that is so predominant in the lower half of an upturning pulvinus. And, the enhanced synthesis of several buffer-soluble and buffer-insoluble proteins as a consequence of gravistimulation may indicate that particular enzymes are being "turned" on by gravistimulation--enzymes such as invertase, beta-glucanase, and glucan synthase, which are key to the processes of cell wall loosening and synthesis that occur differentially in the pulvinus as it bends upward in response to gravistimulation. Calcium may also be an important component of such processes. Our current work suggests that calcium channels play a significant role in cereal grass gravitropism.

Publications

Brock, T.G., Song, I., and Kaufman, P.B. Calcium Ions Do Not Inhibit the Gibberellin-Induced Growth of Internodes of Oat (*Avena sativa* L.) (Abstract). Plant Physiology 80(4, Suppl.): 116, 1986.

Kaufman, P.B., Brock, T.G., and Song, I. Plant Growth Regulators. In: Plant Physiology - A Treatise (ed. by F.C. Steward and A.D. Krikorian). New York: Academic Press, pp. 1-100, 1987.

Kaufman, P.B., Song, I., and Ghosheh, N. Role of Starch Statoliths in the Upward Bending Response in Gravistimulated Barley Leaf-Sheath Pulvini (Abstract). Plant Physiology 80(4, Suppl.): 8, 1986.

Rood, S.B., Kaufman, P.B., Abe, H., and Pharis, R.P. Gibberellins and Gravitropism in Maize Shoots. Plant Physiology 83: 645-651, 1987.

Song, I., Parker, L., Teklinski, A., Kaufman, P.B., and Ghosheh, N. Physiological Changes and Rice Proteins in Seedlings and in the Aleurone Layer Induced by Heat Shock (Abstract). Plant Physiology 80(4, Suppl.): 121, 1986.

CELLS, EMBRYOS AND DEVELOPMENT IN SPACE/MORPHOLOGY OF PLANT CELLS IN SPACE

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Description of Research

It is now generally recognized that the problems of development constitute a major part of the objectives of modern biology. The ultimate aim of our research plan is to furnish systems at different levels of initial organization that will enable the effects of microgravity in the space environment to be tested on the behavior of plants, in contrast or comparison to their performance at 1 g and in ground controls. While the main focus, is aimed towards the broad effects of near zero gravity that operate on systems as they grow and develop, the systems in being are or will be adaptable to a variety of more specific tests. For example, any one of our test systems is capable of being used to ascertain whether there might be differences in the normal rate, frequency and patterning of cell division, or in the fidelity of partitioning of the chromosomes of their cells during or after exposure to spaceflight.

The thrust of the investigations deals with:

(1) The induction of active growth, cell proliferation and metabolism in otherwise mature quiescent cells as they exist in situ. This is a problem that has involved and still involves the identity and mode of action of relatively simple growth regulating substances of low molecular weight, their synergists and cofactors.

(2) The obtaining and multiplication in culture of free cells and their contrasted development into unorganized callus masses, and as somatic (non-zygotic) embryos into plantlets.

(3) The growth, morphogenesis and metabolism of intact plantlets and tissue culture-derived propagules with their established growing regions of shoot and root, in response to interacting factors which are both environmental (i.e., different regimes of photoperiodicity and changing temperatures) and nutritional.

(4) The development of protocols which have a high level of reliability for establishing chromosomal characteristics and profiles for the plant species we are working with, while at the same time seeking to extend the principles so gained to a still broader range of species.

(5) The management of cultured systems from the perspective of being able to use them effectively and with a minimum of human intervention in a space environment setting.

Accomplishments

(1) Streamlined methods have been worked out in which daylily cells in suspension can be sampled and analyzed highly effectively as to their chromosomal profiles anywhere from 7 to 15 days after subculture.

(2) The derivation of cultured cells of daylily which become tetraploid in sustained culture has been characterized from the perspective of the alternative pathways possible.

(3) The management of daylily plantlets by use of ancymidol (cyclopropyl -- (4-methoxyphenyl)-5-pyrimidinemethanol) without adverse effects on clonal growth has been achieved.

(4) Methods have been worked out in which cultivated carrot tissue can be induced to form somatic embryos without the usual use and intervention of growth regulators.

(5) Methods have been worked out in which the chromosomal profile(s) of carrot tissue and roots can be reliably studied using a straightforward protocol for karyotyping.

Significance of the Accomplishments

(1) The value of accomplishment 1 centers not only on the increased reliability of the newly developed methods for a laboratory operation generally requiring a great deal of skill, but on the fact that relatively short-term cultures about a week or so old can now be worked with. Usually 2-3 week-old cultures are best used for examination and this curtails full exploitation of the system for spaceflight tests. The shorter-duration cultures make it totally realistic to use daylily for short as well as long-term space experiments.

(2) The tendency for cultured plant cells to deviate from a usual chromosomal complement profile as a culture ages has been reasonably well documented for several species. In daylily, diploids can become tetraploids. Since tetraploids are larger and generally more attractive, this has been seen as an advantage in those cases where polyploidy is wanted. Even colchicine is unable to convert diploids effectively at 100% level to tetraploidy and, when it occurs, the tetraploid state is not necessarily stable. We have shown that endoreduplication is a significant way whereby tetraploid cells and plants can be generated from cultured cells. Cytomyxis (the movement of genetic material, i.e., chromosomes from the cytoplasm from one cell to the cytoplasm of another) has recently been encountered by us in daylily cells. This peculiar and exciting mode of movement of DNA from one cell to another opens up a whole avenue of interpreting chromosomal profiles in a given culture. It also provides us with another means or parameter of describing and characterizing the chromosomal states in our cultures. This means, in short, that we have another parameter or event with which to "score" cells in space.

(3) Ancymidol has been used in the horticultural industry to keep plants short. The use has been predominantly with dicotyledonous plants. Daylily, a monocotyledon, has been shown to be better manageable in vitro with ancymidol since it keeps

the plantlets compact, and the normally elongated, strap-shaped or "grass-like" leaves can get a bit out of hand in routine culture operations. The use of ancymidol not only keeps plants compact and more discrete, it provides an effective means of germ plasm storage by minimizing and even eliminating the need for frequent transfer of potentially overgrown cultures.

(4) The usual procedure for inducing somatic embryos of higher plants is to employ various growth regulators, usually auxins. Following an initial and generally lengthy exposure to low levels of hormones, careful selection of cell types and subsequent removal of the growth regulator generally leads to formation of non-zygotic embryos capable of growing into mature plants. The long lead time and the use of hormones has made the carrot system a slow one to initiate, albeit a good one to work with once it is in hand. A series of experiments has now shown beyond all doubt that somatic embryos of carrot can be initiated de novo within a couple of weeks without exogenous growth hormones. This allows the system to be initiated more quickly and to be studied in a more nearly normal physiological state.

(5) Carrot has been shown time and again to be a very useful plant from the perspective of developmental and physiological studies. One drawback has been, however, that its chromosomes are very small and difficult to work with. Use of colchicine has not been very successful up until now. The new procedure (using colchicine) is highly effective and provides us with a means of using carrot every bit as effectively as so-called model plants.

Publications

Cronauer, S.S. and Krikorian, A.D. In vitro Re-initiation of Vegetative Growth from Floral Apices of Musa (Abstract). In: Sixth International Congress of Plant Tissue and Cell Culture, Abstracts (ed. by D.A. Somers, et al.), University of Minnesota, Minneapolis 3-8 August, 1986. Minneapolis: University of Minnesota Press, p. 179, 1986.

Cronauer, S.S. and Krikorian, A.D. Regeneration in Bananas. In: Cell Culture and Somatic Cell Genetics of Plants (ed. by I.K. Vasil). New York: Academic Press, Volume 3, pp. 170-186, 1986.

Krikorian, A.D. Clonal Propagation In vitro. (Propagacion Clonal In vitro). In: Fundamentals and Applications of Tissue Culture in Agriculture (ed. by L. Mroginski and W.M. Roca). Cali: Centro Internacional de Agricultura Tropical (CIAT), 1987.

Krikorian, A.D. El Cultivo de Tejidos en el Tropica, Desafio y Perspectivas. In: Primer Congreso Nacional de Cultivo de Tejidos Vegetales (Memorias) (ed. by M. Perea Dallos and Z. Angarita). Bogota: Universidad Nacional de Colombia (Asociacion Colombiana de Estudios Vegetales In Vitro-ACEVIV), pp. 209-239, 1986.

Krikorian, A.D. Genotypic Stability in Cultured Cells, Tissues and Culture-derived Plants. In: Fundamentals and Applications of Tissue Culture in Agriculture (ed. by L. Mroginski and W.M. Roca). Cali: Centro Internacional de Agricultura Tropical (CIAT), 1987.

Krikorian, A.D. and Kann, R.P. Multiplication of the Chinese Tallow, Spaium sebiferum. In: Tissue Culture in Forestry (ed. by J. Bonga and D.J. Durzan) Volume 3. Hague: Martinus Nijhoff/W. Junk, pp. 357-369, 1987.

Krikorian, A.D. and Kann, R.P. Regeneration in Lilies, Irises and Tulips. In: Cell Culture and Somatic Cell Genetics of Plants (ed. by I.K. Vasil) Volume 3. New York: Academic Press, pp. 187-205, 1986.

Krikorian, A.D. and Scheld, H.W. Plant Cell Technologies in Space: Background, Strategies and Prospects. Presented at Space Bioreactor Workshop, Clearlake, Texas, August 22-23, 1985. National Aeronautics and Space Administration Technical Memorandum, 1987.

Krikorian, A.D., Smith, D.L., and Kelly, K. Hormones in Plant Tissue Culture and Propagation. In: Plant Hormones and Their Role in Plant Growth and Development (ed. by P.J. Davies). Hague: Martinus Nijhoff/Dr. W. Junk. p. 1087, 1987.

Mroginski, L., Roca, W., and Krikorian, A.D. Establecimiento de Cultivos in vitro. In: Fundamentals and Applications of Tissue Culture in Agriculture (ed. by L. Mroginski and W.M. Roca). Cali: Centro Internacional de Agricultura Tropical (CIAT), 1987.

Smith, H.W. and Krikorian, A.D. A High Frequency Somatic Embryogenic Carrot System Autotrophic for Exogenous Auxin and Cytokinin (Abstract). In: Sixth International Congress of Plant Tissue and Cell Culture, Abstracts (ed. by D.A. Somers, et al.) University of Minnesota, Minneapolis, 3-8 August, 1986. Minneapolis: University of Minnesota Press, p. 368, 1986.

SENSING AND TRANSDUCTION OF GRAVITROPISM IN PLANTS

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The orientation of plants with respect to gravity is a feature of very significant survival value, as it guides the root system into the soil and directs the shoot system away from the soil. The overall phenomenon has been of interest to experimental biologists since the time of Charles Darwin, but the mechanisms by which such orientation is achieved have been difficult to unravel. Until recently, most research on plant gravitropism has been concerned with either the sedimentation of amyloplasts (starch-filled organelles which sediment in response to gravity) or the redistribution of growth regulating hormones. The research in this project has been aimed at characterizing the first two presumed components of gravitropism: the sensing step and the transduction of the sensing into a physiological gradient across the plant axis. We are using the corn root as the experimental material.

Description of Research

Our first objective has been to seek a physical indicator of gravity sensing. If we can detect the completion of the gravity sensing step -- the first presumed component of gravitropism -- we should have a better capability for characterizing sensing without the confusing involvement of transductive and then response components. We have pursued this objective using a vibrating electrode to measure electric current densities around the root cap during sensing. We have been able to show that there is a shift in electric current density around the sensing organ, the root cap, at the site of sensing and with the same time requirement as we had calculated for the sensing step.

Our second objective has been to find an experimental means of studying the transduction step in gravitropism. If we can experimentally influence the transductive step, we should markedly improve our capacity for unraveling this sector of gravitropism. We have found that we can drive the transductive step with red light. More explicitly, we can expose corn roots to red light and cause them to change from a plagiotropic (seeking the horizontal position) into a positive gravitropic state (seeking the orientation toward the Earth). This finding provides us with an experimental approach to transduction. It permits us to study biochemical events which are related to the establishment of a physiological difference between the two sides of a gravity-stimulated organ.

A third means of studying gravitropism could utilize genetic mutants which have altered gravitropic response characteristics.

We have developed some corn mutants with altered root gravitropism for this part of the study and we are seeking others. Mutants should allow one to learn about the components of a complex physiological response system such as gravitropism by investigating the nature of the mutant limitation.

Accomplishments

In the past year we have made some interesting progress in each of the three areas.

From the examination of the electric current changes with gravity sensing, we have found that changes do occur and occur at the precise locus of gravity sensing with the same timing as is required for the completion of sensing; we have also found that we can selectively inhibit sensing with inhibitors of the cellular regulator, calmodulin. Several other workers in the Space Biology Program have developed convincing evidence that calmodulin plays some important role in gravitropism, but the nature of that role has not been identified. We can show that calmodulin plays an essential role in gravity sensing by the corn root. We also found that we can apply other inhibitors of gravitropism, which are presumed to act selectively, on the growth regulator or hormone systems, and these do not suppress the sensing step as measured by current densities. Thus, we can separately regulate sensing and subsequent components of gravitropism.

From the examination of red light regulation of gravitropism we have made some progress in understanding the transductive processes. First, we have found that calcium and calmodulin play important roles in transduction, for if we use poisons which selectively suppress these factors, we can suppress transduction. Further progress has been made using experiments which attempt to substitute for the red light effect on gravitropism. We have found that serotonin, a neurohumor in animal systems, can substitute for the red light treatment. In animal systems, serotonin acts to activate a second-messenger system called the phosphoinositol-trisphosphate system. This system serves to regulate the calcium content of cytoplasm. We are currently searching for evidence that this second messenger system plays a critical part in gravitropic transduction.

These experiments collectively represent an effort to identify specific biochemical components and events which participate in plant gravitropism.

Significance of the Accomplishments

The overall characteristics of gravitropism in plants are common knowledge; but the biochemistry of the growth regulating systems is complex and uncertain. Several new breakthroughs have identified calcium and calmodulin as important components of the gravitropic complex. By concentrating on separations of the

three major components of gravitropism: sensing, transduction, and growth response, we feel that we are making novel progress in putting the puzzle of gravitropism together. Our definition of some distinctive characteristics of the biochemistry and biophysics of the sensing and transduction steps should organize the pieces of the gravitropism puzzle.

Publications

Bjorkman, T. and Leopold, A.C. Electric Current Changes Associated with Gravity Sensing in Corn Roots (Abstract). Plant Physiology 80(4, Suppl.): 26, 1986.

Sack, F.D., Suyemoto, M.M., and Leopold, A.C. Amyloplast Sedimentation and Organelle Saltation in Living Corn Columella Cells. American Journal of Botany 73: 1692-1698, 1986.

MECHANICAL STRESS REGULATION OF PLANT GROWTH AND DEVELOPMENT

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Description of Research

Research in this laboratory investigates the physiological basis whereby plants respond to mechanical agitation in the growth environment. Mechanical stresses generally are classified according to the manner in which they are applied to plants, e.g., seismic (shaking), thigmic (rubbing), or vibration. At the Earth's surface, wind action, precipitation, and the passage of machinery and animals induce mechanical stresses. Reductions in plant growth and productivity result from these mechanically-induced stresses relative to those in protected areas.

Mechanical stress is not necessarily detrimental to plant growth. Terrestrial plants respond to physical disturbances by acquiring a more compact growth habit. Stems often are shorter, thicker, and stronger. Leaves are smaller and thicker, while water is conserved by closure of stomates and reduced leaf area. However, there are negative effects associated with mechanical stress. Stomatal closure also reduces the amount of CO_2 assimilated by the leaf, thereby reducing photosynthetic productivity and subsequent growth. The onset of reproductive growth often is delayed as is the number of reproductive structures initiated and retained by the plant. Those reproductive structures retained to maturity generally are smaller, compared with those found on non-stressed plants, resulting in a net reduction in harvestable yield.

Dynamic physical disturbances received by plants growing in a spacecraft are not typical of those growing in the terrestrial biosphere. During the first 8 minutes of a space shuttle launch (the time to main engine cutoff), growing plants will perceive acute seismic and vibrational signals. Mechanical agitation of this type likely will retard plant growth for several days relative to that of Earth-based undisturbed controls. Once on orbit, plants are suddenly exposed to a microgravity environment. It is not yet known whether normal instrument operation, astronaut activity, or spacecraft maneuver will be perceived by growing plants as mechanical and/or gravitational stimuli. Is it possible to condition or pre-adapt plants against undesirable growth responses by application of controlled mechanical stimulation prior to launch or on orbit?

The objective of our ground-based research program is to characterize the physiological basis of plant growth responses to brief, periodic episodes of mechanical agitation. Recent

emphasis has focused upon designing model plant bioassay systems useful in investigating the biophysical and biochemical nature of mechanically-induced stress growth responses, cell wall biochemistry, calcium physiology, and the influence of irradiance level upon the degree of mechanical response.

Accomplishments

(1) A model system for the investigation of mechanically-induced stress growth inhibition has been developed using dark-grown soybean seedlings. Intact seedlings were more responsive to brief thigmic (rubbing) stress than to seismic (shaking) stress. This difference may result from the focus of thigmic energy directly on the region most sensitive to mechanical agitation, i.e. the apical hook of the seedling hypocotyl.

(2) Calcium has a small but significant role in mechanically-induced growth inhibition. Exogenous calcium mimics the thigmic stress response and the Ca-induced growth inhibition may be reversed by equimolar concentrations of EGTA (a specific chelator of Ca). However, exogenous EGTA only partially negates thigmically-induced growth reduction in dark-grown seedlings (about 15%). The inability to completely reverse thigmic growth reduction infers the contribution of other physiological processes to the dwarfing response.

(3) There is evidence that calmodulin is involved in the Ca-mediated portion of the mechanical stress dwarfing response. Calmodulin is a calcium-binding regulatory protein found in both plants and animals and is responsible for mediating the activity of Ca-sensitive enzymes. Two putative inhibitors of calmodulin activity, chlorpromazine and 48/80, partially negated thigmically-induced growth inhibition (about 20%). It is not presently known whether the action of these two calmodulin activity inhibitors are physiological or pharmacological. A survey of other inhibitors of calmodulin activity currently is underway to further elucidate the potential involvement of calmodulin activity in the Ca-mediated portion of the mechanically-induced stress dwarfing response.

(4) The level of irradiance significantly affects the degree to which light-grown plants respond to mechanical stress. Experiments in which soybeans were grown in a controlled environment room on benches draped with different densities of shade cloth indicated that plants were much more responsive to mechanical stress at low photosynthetic photon fluxes (PPF) ($150-165 \mu\text{moles s}^{-1} \text{m}^{-2}$, 400-700 nm). At PPF above about $570 \mu\text{moles s}^{-1} \text{m}^{-2}$, seismic stress treatments (280 rpm on a gyrotory shaker 5 min 2X per day) were ineffective reducing growth or dry weight accumulation. Statistically significant, mechanically-induced stress growth responses were observed at $\text{PPF} \leq 315 \mu\text{moles s}^{-1} \text{m}^{-2}$. Since the radiation levels available in the current configuration of the Space Shuttle Plant Growth Unit (PGU) are less than 5% of sunlight level (about $100 \mu\text{moles s}^{-1} \text{m}^{-2}$), sensitivity of plants to mechanical perturbation should be high.

Significance of the Accomplishments

Development of sensitive, rapid, and reproducible bioassay systems (finding #1) will facilitate our investigation of plant response mechanisms to mechanical agitation in an efficient and inexpensive manner. Gaining an understanding of the physiological factors mediating mechanical stress growth inhibition (findings #2 and #3) may result in chemical or physical methods of mimicking, negating, or preadapting plants to detrimental effects of mechanical stress. The confounding effects of dynamic physical agitation on plant gravitropic responses also may be reduced.

Radiation level strongly influences the mechanical stress growth response (finding #4). Modification of the light environment, under which plants are grown on orbiting spacecraft, may prove useful in negating or preventing undesired effects of mechanical agitation.

Publications

Brooks, C.A. and Mitchell, C.A. Sham-stimulated Germination of Waldmann's Green Lettuce Seeds is Related to Weakening of the Endosperm (Abstract). Plant Physiology 80(4, Suppl.): 28, 1986.

Cueller, M.D. and Mitchell, C.A. Effects of Static Versus Flowing Atmospheres on Plant Growth in the Space Shuttle Plant Growth Unit (Abstract). Plant Physiology 80(4, Suppl.): 7, 1986.

Jones, R.S. and Mitchell, C.A. Importance of Calcium in the Mechanically-induced Dwarfing of Soybean. Agronomy Abstracts 97, 1986.

Latimer, J.G., Pappas, T., Mitchell, C.A. Growth Responses of Eggplant and Soybean Seedlings to Mechanical Stress in Greenhouse and Outdoor Environments. Journal of the American Society for Horticultural Science 111: 694-698, 1986.

HOW ROOTS RESPOND TO GRAVITY

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Description of Research

The goal of our research is to determine how roots respond to gravity. We want to understand how environmental signals are translated by plants into predictable growth responses.

The specific goals of the research are:

(1) To quantify how gravity changes the amounts and movement of endogenous elements (including calcium) in graviresponding roots. We accomplished this objective by using microanalysis to examine ultrathin cryosections of cryofixed roots.

(2) To determine how electrical asymmetries affect the movement of endogenous calcium in roots. We accomplished this objective by using atomic absorption spectrophotometry to quantify the amount of calcium in roots growing in electrical fields.

(3) To separate calcium movement from gravicurvature. We accomplished this objective by slowing root curvature with cold temperatures, and concurrently measuring the amount of endogenous calcium with atomic absorption spectrophotometry.

(4) To determine if gravity-induced movement of calcium occurs primarily through the symplast or apoplast. We accomplished this objective by measuring the amount of endogenous calcium in mucilage of graviresponding roots.

(5) To determine the influence of microgravity on cellular structure. We accomplished this objective by continuing the analysis of our experiment flown aboard flight 61-C of the space shuttle Columbia.

Accomplishments

(1) Gravity induces a downward movement of endogenous calcium across the cap and elongating zone of horizontally oriented roots. The resulting gradient of calcium is most pronounced in the mucilage, and cell wall.

(2) Electrical asymmetries that induce gravitropic-like curvature also induce an accumulation of calcium along the concave side of the root.

(3) Gravity-induced accumulation of calcium along the lower side of horizontally oriented roots occurs prior to the onset of gravicurvature.

(4) Microgravity exerts a significant influence on the structure of plant cells. Microgravity tends to increase the relative volume of lipids and cytosol, and tends to decrease the

relative volume of mitochondria, dictyosomes, and plastids in cells of root caps.

Significance of the Accomplishments

(1) Horizontally oriented roots respond to gravity by transporting endogenous calcium to their lower sides. Most of this calcium accumulates in the apoplast, and especially in the mucilage covering the root.

(2) The electrical asymmetries that occur when roots are oriented horizontally may be instrumental in moving calcium to the lower side of roots.

(3) Movement of calcium to the lower side of roots is probably not the result of the differential growth that produces gravicurvature. Rather, our results are consistent with this calcium gradient being responsible for gravicurvature.

(4) Microgravity alters how cells partition their volumes for various activities -- i.e., microgravity alters the structure and function of cells. It decreases the relative volumes of energy-transforming organelles, alters metabolism of starch and lipid, and decreases secretion.

Publications

Evans, M., Moore, R., and Hasenstein, K.-H. How Roots Respond to Gravity. Scientific American 255: 112-119, 1986.

Moore, R., Cameron, I., Hunter, K., Smith, N. and Olmos, R. The Subcellular Concentrations of Endogenous Ions and Elements in Cells of Graviresponding Roots of Zea mays (Abstract). Plant Physiology 80(4, Suppl.): 26, 1986.

Moore, R. and Evans, M. How Roots Perceive and Respond to Gravity. American Journal of Botany 73: 574-587, 1986.

Moore, R. and Fondren, W.M. Possible Involvement of Root-cap Mucilage in Gravitropism and Calcium Movement Across Root Tips of Allium cepa L. Annals of Botany 58: 381-387, 1986.

Moore, R., Fondren, W.M., Koon, E.C., and Wang, C.-L. The Influence of Gravity on the Formation of Amyloplasts in Columella Cells of Zea mays L. Plant Physiology 82: 867-868, 1986.

Moore, R., Fondren, W.M., and Marcum, H. Characterization of Root Agravitropism Induced By Genetic, Chemical, and Developmental Constraints. American Journal of Botany 74: 329-336, 1987.

Moore, R. and McClelen, C.E. Cytochemical Localization of Calcium in Peripheral Cells and Differentiating Epidermal Cells of Primary Roots of Zea mays (Abstract). Plant Physiology 80(4, Suppl.): 26, 1986.

Moore, R., Wang, C.-L., Fondren, W.M., and McClelen, C.E.
Botanical Research Aboard the Space Shuttle: Dynamics of Root
Growth in Microgravity (Abstract). Plant Physiology 80(4,
Suppl.): 26, 1986.

ROLE OF AUXIN IN NORMAL AND ABERRANT (WRONG-WAY) GRAVITROPISM OF TOMATO SEEDLINGS

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Description of Research

A major goal of this laboratory is to contribute to the elucidation of the entire process of gravitropism at a biophysical and biochemical level, that is, to dissect the mechanisms by which plants detect and orient with respect to gravity.

Over a year ago we fulfilled the goals explicit in our proposal on the study of auxin transport (signal transmission), and since then we have been working on a model for gravity reception.

Accomplishments

(1) Development, with K.L. Edwards, of a new model for primary energy focusing and primary energy transduction in gravitropism.

(2) Development, with K.L. Edwards, of a new model for auxiliary energy focusing in gravitropism.

(3) Demonstration, with K.L. Edwards, that the proposed model for primary energy focusing and transduction works in a simple plant sensory system putatively related to the gravitropic system.

(4) Extension of proof that starch statoliths are not involved in gravitropic gravity reception by a representative dicot.

(5) Preliminary characterization of a structure postulated to participate in auxiliary gravitropic energy focusing.

(6) Synthesis of a compound specially designed to aid in the purification of proteins critical for gravitropism.

Significance of the Accomplishments

Signal Transduction: Primary Steps. As described in last year's 1985-1986 NASA Space/Gravitational Biology Accomplishments, Professor Kathryn L. Edwards has been on leave from Kenyon College in order to collaborate on an investigation of a putative role for ion channels in gravitropic reception. We postulated that stretch-activated (s-a) calcium channels of the type described for myoblasts by Guharay and Sachs in 1984 are responsible for transduction of the gravitational signal from a mechanical to a chemical message. The energetics of signal detection suggest that gravity reception is one of the plant's most elaborately evolved sensor systems, and therefore it seemed appropriate to begin our search for plant channels in a simpler sensory system. In particular, the postulated gravitropic

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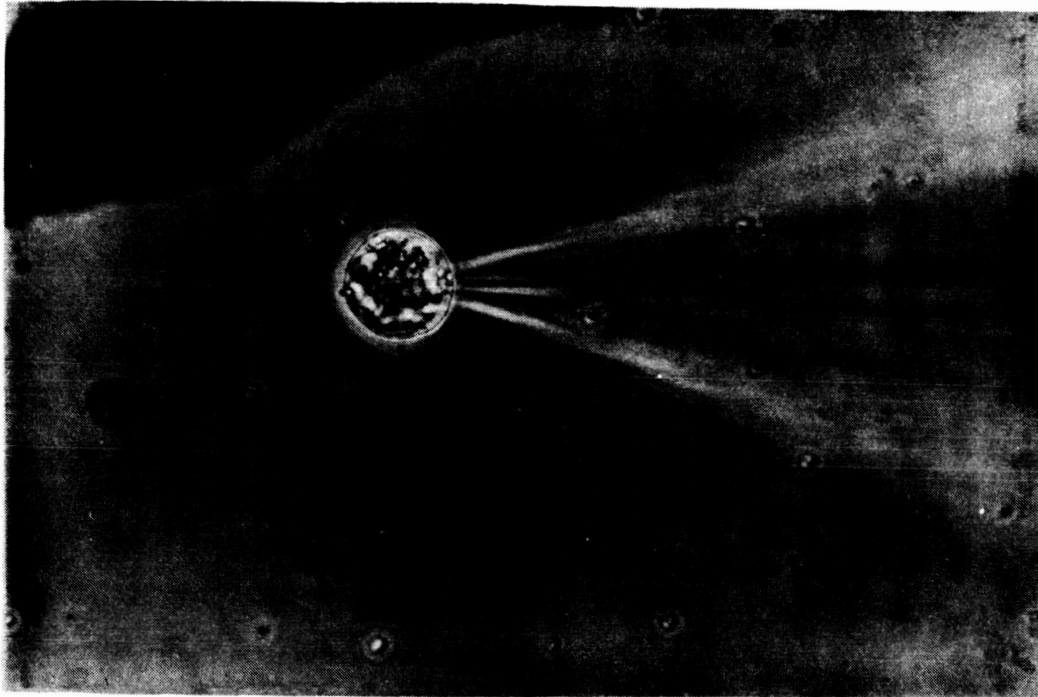


Figure 1. Photograph of a patched protoplast prepared from a suspension culture of tobacco pith cells. The spherical protoplast is gently sucked to the mouth of a heat-polished pipette, and the membrane adheres tightly to the glass rim. Current passing through the patch is recorded via an electrode leading to an amplifier.

TRACE RECORDINGS OF PLANT MEMBRANE ION CHANNELS

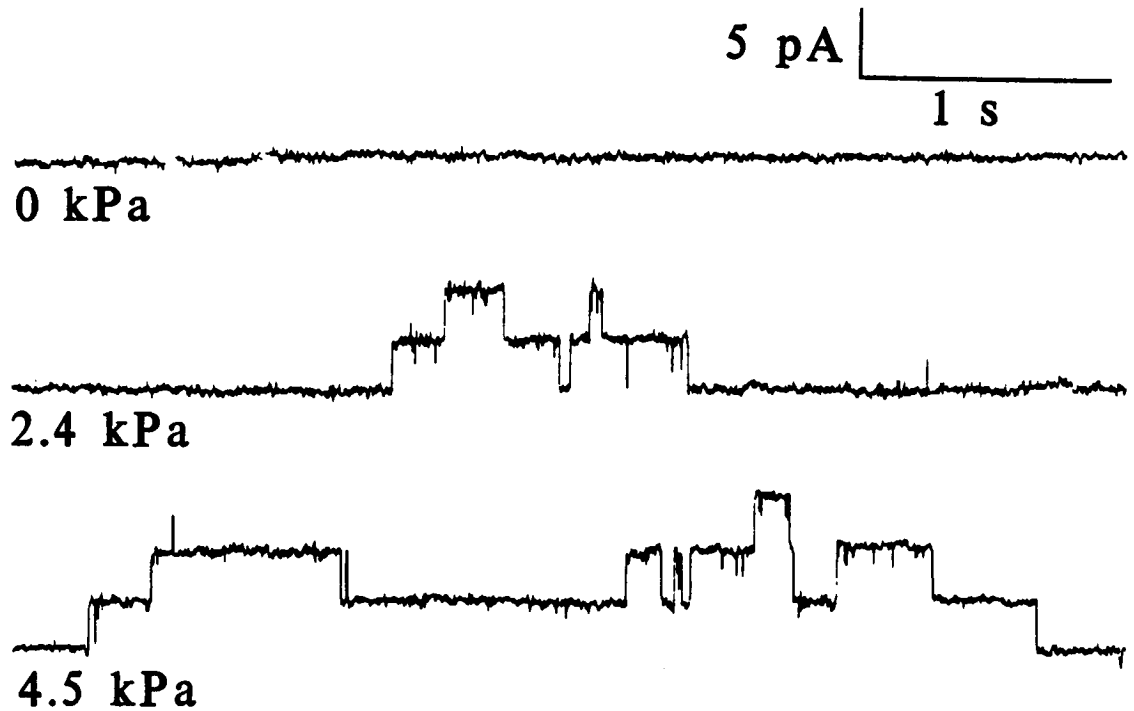


Figure 2. Trace recording of discrete pulses of current that occur when the membrane of a patched protoplast is subjected to gentle suction or pressure. It has been proposed that the transductive step of perception of gravity and other physical stimuli by plant cells is the passage of calcium ions into the cytoplasm through ion channels in the cell membrane, which open in response to mechanical stress deriving from the stimulus. The pulses shown here represent the opening of such mechanically activated ion channels. The amounts of pressure applied, in kilopascals (kPa), are shown.

calcium fluxes are small, and calcium channels are generally more difficult to study than channels that pass bulk ions. S-a channels are typically more difficult to study than other kinds, and as yet no superb methodology for preparing plant membranes for the requisite patch clamping techniques have been worked out. Accordingly, with the collaboration of Stanley Misler and Lee Falke at the Washington University Medical School, we sought high-conductance s-a channels in cultured tobacco cells (Figures 1 & 2). We anticipated finding s-a channels which might be associated with thigmomorphogenesis, with osmoregulation, or with alignment of cell division. In last year's report, we discussed the existence of a high-conductance s-a anion channel. However, we did not mention that we also had several recordings which suggested the existence of a high-conductance s-a cation channel; the recordings were not extensive enough to constitute proof. Edwards has now made more extensive recordings which appear to verify the existence of the cation channel. The plant protoplast membranes have proven hard to patch and additional data are still needed. Specifically, we wish to obtain (a) current-voltage plots with considerably more points per patch, (b) more extensive studies of channel selectivity, and (c) more pressure-response characterizations. The discovery of the second channel is nevertheless a landmark because, given the side-by-side occurrence of high-conductance s-a anion and cation channels, it is clear that the pair will function to detect increases in turgor and effect downward osmoregulation. Turgor regulation would appear to be an excellent model system for the study of gravitropism. More generally, assuming the still-quite-new results can be confirmed and extended, a spin-off of our research on gravity detection will be demonstration of one of the fundamental ways in which plants adapt to osmotica and water tensions in their environment. Because inadequacy of water supply is one of the world's greatest agricultural problems, such adaptation is of great practical importance, and we are in communication with a lab using the same kind of cultured cells to study development of drought-resistant crop plants.

Meanwhile, Edwards is patch clamping protoplasts of the root cap, hoping to find stretch-activated calcium channels which might be transducers of the gravitropic stimulus.

Signal Transduction: Energy Focusing. The s-a channel system effects a considerable focusing of mechanical energy: the s-a channels described above, like the s-a channel originally described by Guharay and Sachs, is sensitive to signals well below thermal noise. However, the level of sensitivity of the s-a channel system per se is nonetheless inadequate to fully account for gravitropic signal reception, and thus we have proposed an auxiliary energy focusing mechanism (Edwards and Pickard, in press). We feel that the development of an alternative model of gravity reception has been an important accomplishment of our research.

Transduction: Statoliths are Not Involved in a Representative Dicot. Two years ago, our laboratory reported on work funded by independent sources, but nonetheless of relevance to the NASA Space Biology program, regarding the irrelevance of starch-filled amyloplasts for gravity reception in a typical dicot. The work, carried out collaboratively with Timothy Caspar and Chris Somerville at Michigan State University, has employed a single gene mutant of Arabidopsis which lacks phosphoglucomutase and hence cannot make starch. This year, experiments have been extended quantitatively, adding still more evidence that statoliths (in the ordinary sense) do not play a role in gravitropism. The demonstration has also been generalized further by extending it to a new experimental system.

Signal Transduction: Secondary Steps. Work by NASA Research Associate J. Henry Slone, joined by NSF-sponsored research associate Terry Riehl, is continuing: hard-won success in obtaining the compound we designed for preparation of an affinity column to purify NPA binding protein, believed to play an important role in secondary signal transduction, is now being followed by efforts to utilize the column in stabilizing, purifying, and characterizing the protein.

Publications

Edwards, K.L. and Pickard, B.G. Detection and Transduction of Physical Stimuli in Plants. In: The Cell Surface and Signal Transduction (ed. by H. Greppin, B. Millet, E. Wagner). Berlin/Heidelberg/New York: Springer-Verlag, 1987.

Falke, L., Edwards, K.L., Misler, S., and Pickard, B.G. A Mechanotransductive Ion Channel in Patches from Tobacco Cell Plasmalemma. Plant Physiology 80 (4, Suppl.): 9, 1986.

Falke, L., Edwards, K.L., Pickard, B., and Misler, S. A Stretch-activated Anion Channel in Cultured Tobacco Cells. Biophysical Journal 52: 251a, 1987.

Harrison, M. and Pickard, B.G. Auxin Asymmetry During "Wrong-Way" Gravitropic Curvature of Tomato Hypocotyls (Abstract). Plant Physiology 80 (4, Suppl.): 7, 1986.

GEOTROPISM IN ARABIDOPSIS THALIANA: A GENETIC APPROACH

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Description of Research

Our ultimate objective is to achieve an understanding at the molecular level of the mechanism whereby a plant measures and responds to a gravitational stimulus. This project is developing and characterizing a family of mutant lines in Arabidopsis with genetic alterations in specific elements critical for the gravitational response. These lines will permit the identification of the elements in the transduction pathway, and will permit an analysis of the interrelations between the two environmental factors, gravity and light, in controlling the growth/physiology of this plant.

This work is divided into several different aspects. First, a family of mutant strains will be identified with altered shoot geotropism. Second, these mutants will be genetically characterized to determine the mechanism of inheritance and the physical location of the gene within the Arabidopsis genome. Third, the mutants will be biophysically characterized to determine if any are receptor mutants. Fourth, other responses to environmental stimuli (phototropism, root geotropism, etc.) will be measured for each mutant strain to identify the interrelationship between these sensory transduction pathways. Fifth, molecular genetic techniques will be employed to physically isolate the genes associated with critical steps in the gravitropism pathway.

Accomplishments

Considerable progress has been made toward the goal of establishing a genetic system for the study of gravitropism in Arabidopsis.

(1) A screening procedure has been developed and used to identify 105 strains of Arabidopsis thaliana with altered gravitropism.

(2) One mutant strain has been identified with random shoot orientation to a 1 g stimulus, but with wild type shoot phototropism and wild type root geotropism. Based on this mutant, one can conclude that at least one critical element early in shoot gravitropism is not critical for root gravitropism. Measurements of phototropism and root geotropism for the 105 strains with altered shoot geotropism will permit us to assess the degree of interconnection between root and shoot gravitropism.

Significance of the Accomplishments

Finding #1. This collection of Arabidopsis mutants with altered shoot geotropism provides a unique resource for studying the interconnections between the pathways in plants for the perception of environmental stimuli and for identifying the events in gravity reception from perception to response.

Finding #2. The assumption until now in the literature has been that the perception of gravity by shoots and roots is the same. The utility of this genetic approach is demonstrated by the conclusion that geotropism in roots and shoots is separable.

CALCIUM MESSENGER SYSTEM IN GRAVITROPIC RESPONSE IN PLANTS

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Description of Research

Signals such as light, gravity, and hormones control diverse physiological processes throughout the life cycle of the plant. However, how the cell senses these signals and converts them into a response is poorly understood and is a subject of great interest. In animals, it is well documented that cyclic AMP, calcium inositol trisphosphate, and diacylglycerol function as messengers for transmitting and amplifying the message received on the surface of the cells. Attempts to explore the role of cyclic AMP in plants have not provided sufficient evidence for its role as a messenger. Recent investigations have shown that Ca^{++} acts as an intracellular messenger. Calcium has been shown to mediate a number of physiological responses elicited by external stimuli. Calmodulin (a ubiquitous calcium-binding protein), calcium-calmodulin dependent protein kinase(s), and protein kinase C have been reported in plants. Various components of the phosphoinositide pathway have also been shown to exist in plants. The involvement of calcium, calcium-dependent protein phosphorylation, and turnover of inositol phospholipids in signal transduction was investigated using a light-dependent gravity responsive variety of corn (Zea mays L., cv. Merit) as an experimental system. Using this system, our goal has been to study the gravity perception and transduction mechanism in plants.

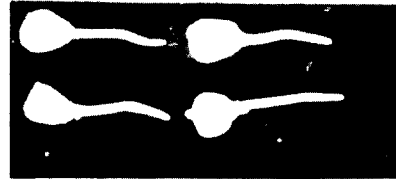
Accomplishments

Our results indicate a major role for calcium in the transduction of extracellular signals such as light and gravity. As shown in Figure 1, depletion of calcium in root tips by EGTA plus calcium ionophore A23187 treatment prior to light treatment resulted in the loss of light-dependent gravisensitivity. Replenishment of calcium to depleted roots restored the light-dependent gravisensitivity. Exposure of root tips to light resulted in marked promotion of phosphorylation of specific polypeptides, and these changes in protein phosphorylation were blocked by calcium depletion prior to light treatment.

Our results indicate that light can promote the hydrolysis of inositol phospholipids which has been shown to result in an increase in cytoplasmic calcium. This increase can trigger a cascade of biological changes by activating calcium-regulated enzymes such as protein kinases which eventually result in a final response. In addition, light treatment of dark-grown roots resulted in an increased level of inositol trisphosphate, as

EFFECTS OF CALCIUM MANIPULATION ON ROOT GRAVITROPISM

1) CONTROL (DARK)

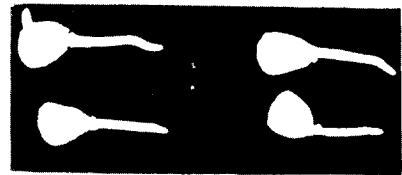


2) CONTROL (LIGHT)



3) EGTA+A23187 (DARK)

↓
(LIGHT)



4) EGTA+A23187 (DARK)

↓
Ca+ A23187 (DARK) → (LIGHT)

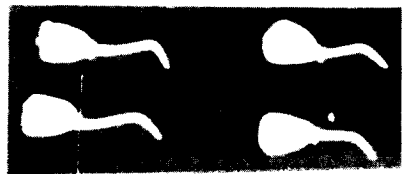


Figure 1. Effect of calcium manipulation on light-induced gravitropic response in roots. Corn seeds were grown for 48 hr in the dark, and the roots were immersed in either buffer alone (1,2) or with 5 mM EGTA and 1 μ M ionophore A23187 (3,4) for 1 hr. Following this treatment, 2 and 3 were exposed to light for 10 min whereas 4 was treated with 1 mM CaCl_2 + 1 μ M A23187 for 30 min prior to 10 min light exposure. The roots were then set horizontally and kept in dark for 6 hr to observe curvature.

compared to control. Furthermore, 5-hydroxytryptamine, which is known to promote the hydrolysis of phosphoinositides, induced gravitropic curvature in dark-grown roots and increased inositol trisphosphate levels.

Significance of the Accomplishments

In view of these results, we suggest that calcium, calcium-dependent protein phosphorylation, and inositol phospholipid turnover may play a key role in signal transduction in plants. These results introduce a new experimental approach to understanding the transduction of extracellular signals in plants. Increased understanding of gravitropic response in plants could help to design life-support systems for space exploration.

Publications

Poovaiah, B.W., Reddy, A.S.N., and McFadden, J.J. Calcium Messenger System: Role of Protein Phosphorylation and Inositol Phospholipids. Physiologia Plantarum 69: 569-573, 1987.

Raghothama, K.G., Reddy, A.S.N., Friedmann, M., and Poovaiah, B.W. Effect of Calcium on in vivo Protein Phosphorylation in Corn Root Tips (Abstract). Plant Physiology 80 (4, Suppl.): 61, 1986.

Reddy, A.S.N. and Poovaiah, B.W. Effect of Phospholipids, Diacylglycerol and Inositol Trisphosphate on Phosphorylation of Proteins from Corn Coleoptiles (Abstract). Plant Physiology 80 (4, Suppl.): 141, 1986.

Reddy, A.S.N. and Poovaiah, B.W. Inositol 1,4,5-trisphosphate Induced Calcium Release from Corn Coleoptile Microsomes. Journal of Biochemistry 101: 569-573, 1987.

Veluthambi, K. and Poovaiah, B.W. In vitro and in vivo Protein Phosphorylation in Avena sativa L. Coleoptiles: Effects of Ca^{2+} , Calmodulin Antagonists and Auxin. Plant Physiology 81: 836-841, 1986.

EFFECTS OF HYPERGRAVITY AND HYPOGRAVITY ENVIRONMENTS ON CELL CYCLE REGULATION DURING FERN SPORE GERMINATION

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Description of Research

It is probable that today's plants evolved under constant gravitational forces. As gravity comprises an integral component of the environmental milieu which supports plant growth, any change in its level could also affect plant growth. The purpose of this research is to study the effects of gravity on a component phase of plant growth.

Anatomically, plant growth is thought to be accomplished by increases in cell number and cell size. Therefore the effects of gravity on cell division (mitosis), or more properly stated, component phases of the cell cycle, are important in understanding the growth of plants in any environment that has a different gravitational force than that of Earth. As growing plant tissue typically consists of cells at various developmental stages, it is important to employ a model system of plant cell division that would allow for the study of the effects of gravity on a single cell as it progresses through the cell cycle. This is necessary so that the effects of gravity, if any, can be studied on the various stages of the division process. Because of this and the need for a large number of cells at a similar developmental stage, spores of the sensitive fern, Onoclea sensibilis, were chosen as the test material. Spores from this plant are collected in a dry dormant state and upon hydration and light activation undergo a mitotic division which leads to germination.

Accomplishments

Results from experiments performed demonstrated that:

(1) The application of hypergravitational forces causes a significant effect on cell division, as evidenced by delays in germination of spores of the sensitive fern.

(2) Various levels of hypergravitational force may affect the component phases of the cell division process differently.

Significance of the Accomplishments

Results from several experiments demonstrate that cell division, hence germination, is significantly delayed after spores of Onoclea sensibilis have been subjected to gravitational stress. These results also show that when spores are stressed with high gravitational forces (e.g., 1,000 to 10,000), the cytoplasm is reorganized and sedimented into definite layers. As this

layering obviously changes the structural and physiological relationships of organelles in the spore, the possibility arises that this disruption causes the delay in cell division and that this delay is more a result of the sedimentation and recovery processes than a direct result of gravity. However, results from a similar experiment using a lower gravitational force level (which does not cause sedimentation) shows that a statistically significant delay in the time taken to complete cell division is observed even when cytoplasmic layering is not evident. Therefore, it appears that hypergravitational stimuli have an effect on some component phase of the cell division process, which ultimately results in a lengthening of the time required for cell division to occur.

Presently, techniques are being worked out to isolate proteins from spores stressed by gravitational forces. This is being done to evaluate if the observed delay in cell division after hypergravitational stimuli involves some change in protein synthesis. Shock proteins have been shown to be involved in delaying the cell division process in many plants. These proteins were shown to develop as a result of various treatments ranging from temperature to pressure. To date we know of no reports linking gravitational stress to the formation of shock proteins. However, based on our observations a search for some type of induced stress protein appears logical.

Also, other work is being planned or has begun in order to test the effects of hypogravitational stimuli on cell division and also to define the length of time required for the spores to complete each of the component phases of the cell cycle. It is hoped that when this work is complete, valuable information can be gained which will show how gravity affects cell division and plant growth.

MECHANISM OF SHOOT GRAVITROPISM

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Description of Research

A basic question in plant physiology is the mechanism by which plants transduce information about the direction of gravity into an oriented direction of growth. Information gained about how plants respond to gravitropic stimulation will enhance knowledge of a basic adaptive mechanism and allow us to better understand and control the orientation of plant organs in the space environment.

Presently, when a shoot is oriented in a horizontal position it begins to curve upward in a smooth arc after about 20-30 min. Reorientation is usually complete within 2-4 hr. This curvature response ultimately derives from enhanced growth of those cells comprising the lower portion of a horizontal shoot and a retardation of cell growth near the upper surface. The goal of this research is to understand the factor or factors responsible for this asymmetric growth. Toward this end, research has centered on the physiological properties of auxin and calcium as potential mediators of gravitropism.

In the past this research utilized Avena coleoptiles and Helianthus hypocotyls as model systems. While these systems were useful, continued progress would be more likely if the utilization of genetic mutants were possible in this research. Thus during the past year our research efforts have shifted to the gravitropic tomato mutant, diageotropica (dgt). This mutant is the result of a single gene mutation which confers a reduced sensitivity to auxin and an extremely low ethylene requirement for normal growth and development. Genetic studies of dgt and its two alleles demonstrate that its prostrate morphology is conditioned by a single base pair substitution resulting in many pleiotropic effects: diageotropy (unsupported horizontal growth) of both shoots and roots, hyponastic leaves, lack of lateral roots, and lack of large xylem vessels in secondary vascular tissues of the shoot. Diageotropica's isogenicity with cv. VFN8 suggests that any response not coded by dgt's single mutant gene should be paralleled by an identical response in the isogenic parent variety and, conversely, any response by dgt not paralleled by a similar response in cv. VFN8 must be due to its single mutant gene. We intend to exploit this characteristic to further study the mechanism of auxin/ethylene action in plant gravitropic responses.

Accomplishments

Gravistimulation experiments with adult dgt plants (3-5 leaf stage) have demonstrated a lack of response after 24 hr, whereas our experiments with dgt hypocotyls (7 days old) have shown a sluggish, but definite response after a 24 hr stimulation. The sluggish gravitropic curvature observed in dgt might be explained by an altered auxin transport mechanism and/or an altered auxin receptor. Preliminary data indicate the rate of auxin polar transport in dgt hypocotyls is very low relative to wild-type controls. The inability of dgt hypocotyl sections to elongate in response to exogenous auxin (but not other growth-enhancing agents) also suggests this mutant has an altered auxin receptor.

While little is known about biochemical similarities and differences between dgt and wild-type plants, some preliminary information is available. SDS-PAGE electrophoresis of VFN8 and dgt proteins reveals no differences in banding patterns. However, as previously reported by Zobel, starch gel electrophoresis reveals a modified (smaller) acid phosphatase isozyme in dgt extracts. We are currently in the process of purifying the mutant and wild-type isozymes for further characterization. In the forthcoming months we will be assaying the purified isozymes (obtained by electroelution from the starch blocks) and comparing wild-type and dgt preparations for phosphatase and ATPase activity.

We have recently established the mutant and wild-type in tissue culture which provides for many future experimental opportunities. Tomato is regenerable from culture, making it a candidate for genetic modification. An interesting prospect with the cultured tomato cells would be to "correct" the mutation by inserting a cDNA clone of the wild-type gene or by inserting an extra auxin gene via the Ti-plasmid of Agrobacterium tumefaciens. Eventually, modifications of this sort will allow us to study, in vivo, the mechanisms of hormonally controlled gene expression.

Significance of the Accomplishments

The overall purpose of our research is to further define the control mechanism of plant cell elongation during gravitropic curvatures. We are presented with a unique opportunity to utilize a relatively unstudied gravitropic mutant and its isogenic control as our model system. Because of dgt's altered auxin/ethylene physiology and its isogenicity with the control, simple comparisons of auxin transport rates, shoot elongation rates, and investigations at the mRNA and protein levels should tell us much about this mutant and the mechanism of gravitropism.

Our findings that dgt seedlings have reduced auxin transport, elongation, and gravicurvature capabilities strongly suggest the dgt lesion is related to an auxin receptor. Our preliminary electrophoresis results indicate that we may be able to

biochemically define the altered gene product. The finding that we can establish both the mutant and wild-type lines in tissue culture provides us with an opportunity to eventually conduct gene modification experiments.

REGULATION OF ENZYMES DURING LIGHT-STIMULATED GRAVITROPISM

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Description of Research

Light greatly accelerates the gravitropic response of roots, coleoptiles, and stems in certain plants. This indicates that some cellular response initiated by light is the same as, or affects, one of the gravity-induced cellular responses necessary for gravitropism. The objective of this research is to try to identify the specific cellular processes that are altered by gravity and light during the induction of gravitropic growth in plants.

The best characterized photoreceptor for light-regulated gravitropism is the pigment phytochrome. The photoconversion of this pigment to an activated form called Pfr accelerates the gravitropic curvature of some shoots and roots. Both Pfr and gravistimulation initiate calcium transport changes in responding cells. Ca^{2+} is now recognized as an important "second messenger" for coupling external stimuli to growth responses in plants and animals. To evaluate whether Ca^{2+} helps to mediate light-stimulated gravitropism, it will be important to identify growth-affecting targets of Ca^{2+} action in cells. Ca^{2+} can affect both wall extensibility and the expression of specific genes, two responses that could certainly impact on growth. These considerations have led us to begin analyzing enzyme activities in walls and nuclei that are controlled by light, especially those that are regulated also by Ca^{2+} .

In corn coleoptiles, the effects of Ca^{2+} on wall extensibility appear to be mediated through the activity of one or more enzymes. However, as yet no enzyme target of Ca^{2+} action in the wall has been identified. Cell wall enzymology in general is poorly understood. As an aid to studying wall metabolism, we have raised a library of 22 monoclonal antibodies (mAbs) against wall antigens and have identified four of them as being against the growth-affecting wall enzymes peroxidase and cellulase. In pea nuclei we have identified a light-regulated enzyme (an NTPase) whose activity probably helps control the rates of messenger synthesis. Last year we reported that the stimulation of this enzyme by light requires Ca^{2+} . This year we have purified the major NTPase in pea nuclei and have found that its activity is strongly stimulated by Ca^{2+} -activated calmodulin.

Accomplishments

(1) We have raised and partially characterized a library of 22 monoclonal antibodies against wall antigens. Most of these

are against wall proteins; we expect that some of them will be against wall polysaccharides. We have determined that four of the mAbs are directed against wall enzymes: two against a wall peroxidase and two against a wall cellulase.

(2) We have carried out immunogold localization studies to confirm that the antiperoxidase and anticellulase mAbs recognize peroxidases and cellulases that are present in the walls of corn coleoptiles. These enzymes are secreted into the wall from cytoplasmic vesicles, and the mAbs tested recognize both the cytoplasmic forms and the wall forms of the enzymes.

(3) We have purified a phytochrome-regulated nuclear NTPase from pea nuclei and have demonstrated that this enzyme is stimulated over 3.5-fold by Ca^{2+} -activated calmodulin.

Significance of the Accomplishments

Finding #1: Each of these antibodies cross-reacts with a specific cell wall component. Their value as a probe will depend to some extent on how important the antigens they recognize are in the control of cell wall metabolism. Peroxidase and cellulase, for example, are important regulatory wall enzymes. The antiperoxidase and anticellulase mAbs will be useful for constructing radioimmunoassays to determine whether light or gravitropic stimuli change the rate of delivery of these enzymes into the wall. They can also be used to perfuse the walls to examine whether antibody binding to these enzymes affects gravitropic growth.

Finding #2 identifies the locale of the antigens recognized by the mAbs. The preparation of "wall antigens" used to inoculate mice for mAb production may have had a cytoplasmic contamination level of 1 or 2%, so some of the mAbs produced could have been directed against cytoplasmic antigens. There are, for example, some isozymes of peroxidase that are not found in the wall. The immunolocalization results confirm that the mAbs tested are against antigens that are present in the walls of corn coleoptiles.

Finding #3 is the first demonstration of a nuclear enzyme that is regulated by Ca^{2+} and calmodulin. It focuses attention on the possibility that gravitropically induced changes in the transport of Ca^{2+} could affect nuclear enzyme activities that impact on RNA synthesis. Recent publications show that both Pfr and hormones promote rapid changes in gene expression that are important for the growth changes induced by these stimuli, so it is appropriate now to investigate whether nuclear activity changes help mediate the rapid growth responses stimulated by light and gravity.

Publications

Chen, Y.-R., Datta, N., and Roux, S.J. Purification and Characterization of a Nuclear NTPase (Abstract). Plant Physiology 80(4, Suppl.): 15, 1986.

Chen, Y.-R. and Roux, S.J. Characterization of Nucleoside Triphosphatase Activity in Isolated Pea Nuclei and Its Photoreversible Regulation by Light. Plant Physiology 81: 609-613, 1986.

Datta, N., Hardison, L.K., and Roux, S.J. Polyamine Stimulation of Protein Phosphorylation in Isolated Pea Nuclei (Abstract). Plant Physiology 80(4, Suppl.): 15, 1986.

Datta, N. and Roux, S.J. Regulation of Enzymes in Isolated Plant Nuclei. BioEssays 5: 120-123, 1986.

Dauwalder, M., Roux, S.J., and Hardison, L. Distribution of Calmodulin in Pea Seedlings: Immunocytochemical Localization in Plumules and Root Apices. Planta 168: 461-470, 1986.

Dauwalder, M., Roux, S.J., Hardison, L.K., and Dedman, J.R. Localization of Calcimedlin in Pea Seedlings by Immunocytochemistry (Abstract). Journal of Cell Biology 103: 453a, 1986.

Roux, S.J. and Dauwalder, M. Distribution and Possible Functions of Calcium and Calmodulin in G-Sensing of Plants (Abstract). In: Twenty Sixth Plenary Meeting of the Committee on Space Research, Abstracts. Paris: COSPAR, p. 330, 1986.

Roux, S.J. and Serlin, B.S. Cellular Mechanisms Controlling Light-Stimulated Gravitropism: Role of Calcium. CRC Critical Reviews in Plant Sciences 5: 205-235, 1987.

CELLULAR POLARITY AND INTERACTIONS IN PLANT GRAVIPERCEPTION

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Description of Research

The ultimate goal of our research is to contribute to the identification of the site of gravity perception in higher plants. Many botanists suspect that heavy, starch-containing organelles, amyloplasts, play an essential role in this process, but it is unclear where in the cell amyloplasts act. Sedimented amyloplasts are only found in a few locations in plants, e.g., in root caps and in a sheath of cells surrounding the vascular tissue in stems. We hope to determine whether amyloplasts interact with the cytoskeleton and with auxin transport carriers in these cells.

The cytoskeleton is composed of different systems of filaments, in turn consisting of different proteins. Our previous observations of cells containing sedimented amyloplasts indicate that gravity-induced amyloplast sedimentation is not straightforward, but is influenced by cytoplasmic streaming in roots and shoots. Cytoplasmic streaming is thought to be mediated by microfilaments. If such a cytoskeletal system were present in cells containing sedimented amyloplasts, amyloplasts could perhaps trigger perception by "tugging" on these filaments or by repositioning membrane components with microfilaments as intermediaries. It is not known whether a microfilamentous cytoskeletal system is present in these cells.

Dr. Mark Jacobs has developed monoclonal antibodies to presumptive auxin transport carriers in pea stems. Using immunofluorescence, he has found that these hormone carriers are located in the cell membrane along the bottom of various cells, including cells that contain sedimented amyloplasts. Because auxin may be involved in inducing gravicurvature, it is important to understand the distribution of these carriers in vertical and horizontal organs. First, however, it is necessary to confirm Dr. Jacob's observations regarding the distribution of labelling produced by his monoclonal antibodies.

Accomplishments

(1) The cells that contain amyloplasts that sediment have an intricate network of microfilaments. These microfilaments were visualized using fluorescence microscopy (Figure 1). In coleoptiles (Figure 1A), the microfilaments are either large, essentially straight cables in large transvacuolar strands, or finer, more sinuous cables found in the cell periphery.

MICROFILAMENTS IN CORN COLEOPTILE AND ROOT CELLS

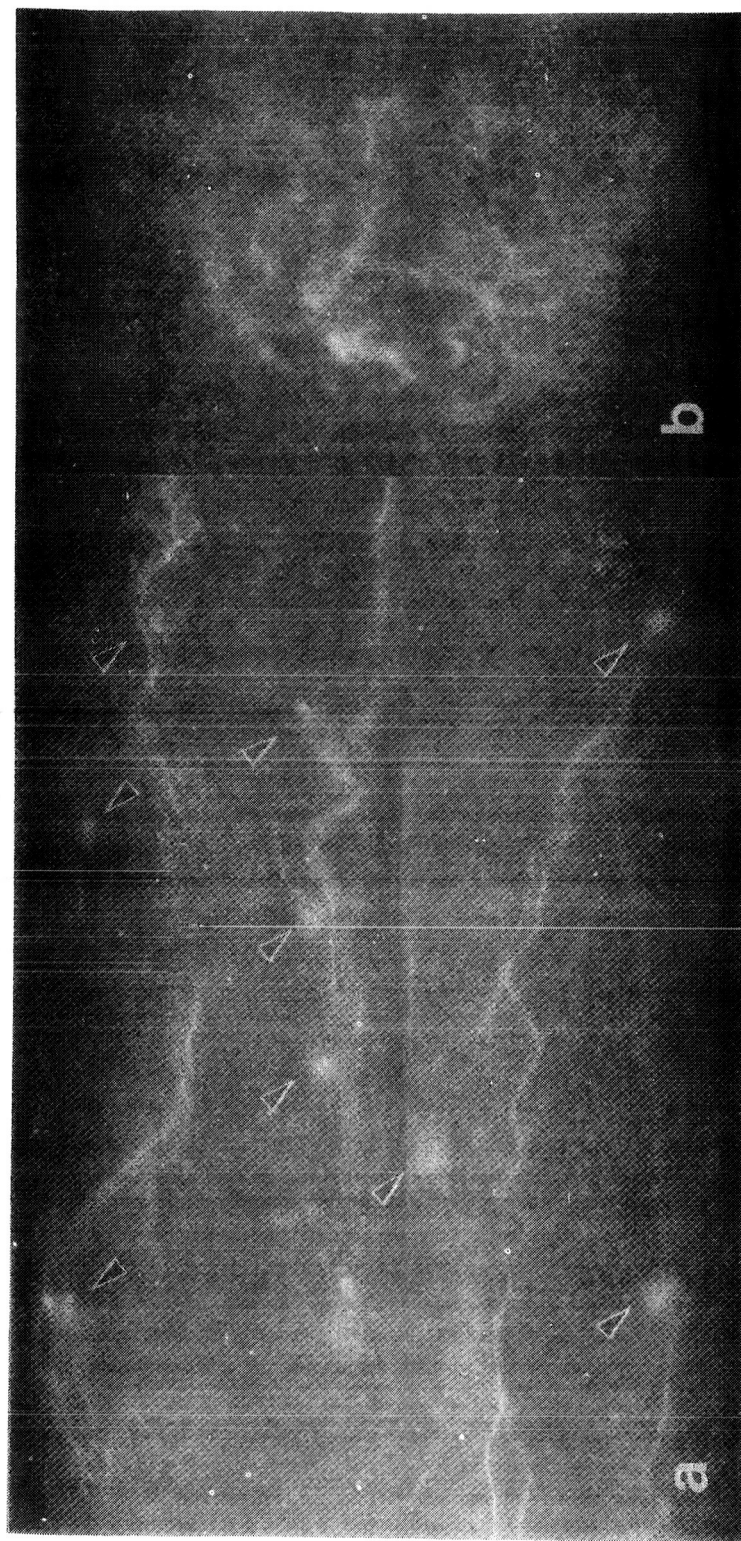


Figure 1. Actin microfilament bundles (bright lines) as visualized by fluorescence microscopy (using rhodamine phalloidin). a. Microfilaments in corn coleoptiles. The amyloplasts (which are autofluorescent) are indicated by darts. Note the numerous microfilaments close to, and in some cases, surrounding amyloplasts. b. Microfilaments in central cells of a corn root cap. The cell isolation procedure caused the loss of the starch in the amyloplasts but did allow the visualization of a reticulate network of microfilaments throughout the cytoplasm.

Microfilaments appear to encircle both the nucleus and the amyloplasts. A system of microfilaments also was found in root cap columella cells (Figure 1B).

(2) In dark-grown pea stems of different ages, the only place where sedimented amyloplasts are consistently found is close to the zone of the stem that curves upward when plants are placed on their side.

(3) We confirm the recent findings of Caspar, Pickard, and Somerville, that mutants of Arabidopsis that completely lack starch also lack sedimented amyloplasts in their root caps, but are fully graviresponsive.

(4) One of the monoclonal antibodies that inhibits NPA binding to membranes specifically labels the nucleus, and another labels the cell wall.

Significance of the Accomplishments

Finding #1. Previous studies of the cytoskeleton in the cells thought responsible for graviperception primarily identified microtubules. Our results suggest that microfilaments are abundant in these cells as well. Whereas both microtubules and microfilaments are found in the periphery of the protoplast, microfilaments are also abundant deeper inside the cell. Some microfilaments surround each amyloplast. Thus, microfilaments are more in a position to interact with amyloplasts than are microtubules. Clearly, however, this positional relationship does not establish a functional connection.

Finding #2. Sedimented amyloplasts are absent throughout the length of long internodes (e.g., 7 inches) of pea stems, except in the zone that retains the ability to curve away from gravity. This is one more piece of correlative evidence that points to the involvement of sedimented amyloplasts in graviperception. However, see #3 below.

Finding #3. We confirm the absence of any sedimented amyloplasts in the root caps of the starchless but gravitropic mutant of Arabidopsis. This shows that sedimented amyloplasts are not necessary for graviperception in all species of higher plants. It remains to be determined how perception can occur in the apparent absence of a cell component with enough mass to be stratified in a cell. Obviously, we also hope to determine whether amyloplast sedimentation is somehow utilized in perception in species in which amyloplasts are present.

Finding #4. We are still in the process of screening different antibodies using various localization protocols. However, to date, the only definitive labelling that we have seen using the monoclonal antibodies provided by Dr. Jacobs is to an antigen located in the cell wall and to another in the nucleus (but not the nucleolus). All the monoclonal antibodies being tested are active in inhibiting binding of NPA to membranes i.e., all the antibodies appear to interact in a specific way either with a chemical that binds to the auxin transport carrier or with the

carrier itself. We are currently exploring what factors might be responsible for antibody binding to such diverse antigens.

Publications

Sack, F.D. Starch Sheath Differentiation in Pea Epicotyls (Abstract). Plant Physiology 80(4, Suppl.): 8, 1986.

Sack, F.D., Suyemoto, M.M., and Leopold, A.C. Amyloplast Sedimentation and Organelle Saltation in Living Corn Columella Cells. American Journal of Botany 73: 1692-1698, 1986.

White, R.W. and Sack, F.D. Actin Microfilaments in the Presumptive Statocytes of Grass Coleoptiles. Journal of Cell Biology 103: 110a, 1986.

GRAVITROPISM IN DICOT STEMS

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Description of Research

When a stem is turned on its side, perception of gravity leads to an almost complete cessation of growth on the top of the stem with a typically accelerated growth rate on the bottom and an upward stem curvature. Sixty years ago when the plant-growth hormone, called auxin (usually indoleacetic acid: IAA), was discovered, it was suggested that stem bending occurred as auxin was transported toward the bottom side of the stem. Both lateral auxin transport and internal auxin gradients have been observed, but there are quantitative difficulties. Compared with the known growth responses of stems to externally applied auxin solutions, measured auxin gradients in the stems seem too small to account for observed growth rates of top and bottom surfaces of a bending stem. No one has ever measured an absence of auxin in the upper tissues, for example, where growth usually stops and shrinkage may even occur.

There is an alternative to the transport hypothesis: The tissues may change in their sensitivity to auxin. Even if auxin remained constant in the tissues, upper tissues might become much less sensitive while lower tissues might increase (or remain about the same) in sensitivity to auxin.

We are investigating the sensitivity hypothesis primarily by two approaches. First, we immerse horizontal seedling stem sections (soybean and sunflower seedlings) in auxin solutions over a wide range of concentrations (seven orders of magnitude) and measure growth rates of upper and lower surfaces. Second, we measure the amount of auxin that penetrates top and bottom tissues from the external solutions and also the active auxin that occurs internally in the tissues.

Accomplishments

(1) Development of techniques to study the kinetics of growth as a function of auxin concentration. During the past year, it became evident that precise measurements were necessary to plot growth rates for upper and lower surfaces as a function of auxin concentrations. We now have two plexiglas tanks, each consisting of eight vertical compartments, in which excised stem segments are held in a horizontal or vertical position. The stems are machine marked with white oil paint, and the tanks with stems are photographed (with flash) at intervals for 6 hr or more by two matched view cameras that use 4x5 sheet film. The negatives are projected and the images measured (bending angles

and surface distances between marks). A calibration marker attached to the holder by each stem segment allows computer calculation of lengths in absolute units. We now have a special projection table; the image is reflected by two mirrors and projected from below onto a screen that is the surface of a digitizer. Data are transferred directly to the computer, where calculations will be made with the help of a spread sheet (yet to be implemented). This will allow us to increase the accuracy of our measurements and obtain data on large numbers of stems so the observed differences can reach a high level of statistical significance.

(2) Results of kinetic studies thus far: In four experiments that were completed before our digitizer became operational, it was evident that extremely low concentrations of auxin (10^{-8} M IAA) promoted growth of lower tissues maximally; higher concentrations led to less instead of more growth of bottom tissues. But top tissues were promoted by much higher auxin levels (10^{-4} M IAA). Sensitivity is inversely proportional to the amount of auxin required to produce a given amount of growth; the tissue is less sensitive if more auxin is required to produce some growth rate. These results strongly suggest that sensitivity of upper tissue to auxin decreases upon gravistimulation. Since this conclusion suggests that the transport hypothesis at least needs modification, it has been challenged by other workers in the field. The only meaningful challenge concerns the internal concentrations of auxin compared with those in the external solutions. Thus, it is essential to measure the amount of auxin that penetrates from external solutions and, in addition, to measure internal concentrations of native plus added auxin as accurately as possible.

(3) Preliminary results of auxin measurements. During late 1985 and early 1986, we measured penetration of 14 C-IAA from external solutions. We found that, after three hours in the solutions, concentrations of 14 C-IAA in the tissue were between 10 and 20% of those in the external solution, this being true for both upper and lower halves and for all concentrations. On the basis of these results, one challenge to our interpretation of the kinetic data has been met: the curvature and growth results cannot be explained in terms of differences in penetration of auxin. It was suggested by the Space Biology Peer Review Panel that we use the Avena-coleoptile curvature auxin bioassay that was developed in 1926. This technique has been supplanted in virtually all modern laboratories by chemical and physical techniques. But in one respect it is better than these techniques: it should also detect unknown molecules that have auxin activity. We have been learning the technique for the Avena-curvature bioassay. We can obtain acceptable calibration curves, and within a few days we should have the first results of our efforts to extract auxin with ether.

Significance of the Accomplishments

Finding #1. We cannot reach our goals without suitable techniques, and development of these is now essentially complete.

Finding #2. We find the growth data to be highly convincing and strongly indicative that sensitivity of tissues toward auxin (probably the tenacity with which auxin is bound to its receptor sites) greatly decreases in upper stem tissues upon gravistimulation. If this conclusion is accepted, it is highly significant, since plant physiology has largely overlooked the possibility that sensitivity changes might account for some plant responses to growth regulators. For sixty years, most research on plant gravitropism has been directed toward research on the auxin-transport hypothesis. Much of this effort may have been misdirected.

Finding #3. The results of studies on penetration of ^{14}C -IAA also strongly support the hypothesis of changing sensitivity in that they do not support the transport hypothesis. So far, we have no results with the Avena-curvature bioassay.

Publications

Salisbury, F.B., Rorabaugh, P., White, R.G., and Gillespie, L. A Key Role for Sensitivity to Auxin in Gravitropic Stem Bending (Abstract). Plant Physiology 80(4, Suppl.): 26, 1986.

Wheeler, R.M., White, R.G., and Salisbury, F.B. Gravitropism in Higher Plant Shoots. IV. Further Studies on Participation of Ethylene. Plant Physiology 82: 534-542, 1986.

White, R. and Salisbury, F.B. Wall Extensibility Changes During Gravitropic Bending in Dicot Stems (Abstract). Plant Physiology 80(4, Suppl.): 26, 1986.

ANIMAL PROJECTS

EFFECT OF SKELETAL UNLOADING ON BONE FORMATION

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Description of Research

The long-range goal of our research program is to understand the effects of gravity on skeletal development and bone metabolism. Our present objectives are to define the effects of gravity or mechanical stress on bone formation and resorption, and to determine the mechanisms (hormonal or paracrine) by which mechanical stress is coupled to bone cell activity.

Bone is a dynamic, living tissue. It continually is undergoing change, or remodeling, which involves a delicate balance between bone formation and bone resorption. This balance is influenced by systemic hormones such as parathyroid hormone, glucocorticoid hormones, and the vitamin D metabolites, as well as local factors such as blood flow, neuromuscular activity, and mechanical stress. Recently, a number of cytokines have been observed to stimulate or inhibit bone formation and resorption. Some of these cytokines, such as somatomedin-C, transforming growth factors, and fibroblast growth factors, have been identified in bone. Such cytokines may participate in coupling mechanical stress to bone cell activity, or the activity of one type of cell to the activity of another type of cell, for example, osteoblast activity to osteoclast activity. Our research over the past year was directed primarily toward elucidation of the role that the systemic hormones play in coupling the mechanical stress of weight bearing to the cellular response of bone formation. Currently we are establishing the procedures required to assess the role of locally produced cytokines.

To evaluate the roles of systemic hormones in the response of bone to skeletal unloading, we have continued to use the hind limb unloading model to produce skeletal unloading in rats. Bone formation was assessed by histomorphometry, ^{45}Ca and ^3H -proline incorporation, as well as change in fat-free weight and calcium content. Bone maturation was assessed by separating different fractions of powdered bone using toluene: bromoform density gradient centrifugation and evaluating the fractions for bone weight, total calcium, and ^{45}Ca and ^3H -proline incorporation. The effect of the active vitamin D metabolite $1,25(\text{OH})_2\text{D}$ on bone formation and mineralization was determined by infusing the $1,25(\text{OH})_2\text{D}$ into the rats with osmotic minipumps. The role of glucocorticoid hormones in mediating changes in bone formation with skeletal unloading was determined using surgical ablation techniques. To initiate studies of locally produced cytokines in the regulation of bone formation and resorption, we established

cultures of mouse calvarial cells and long bone chondrocytes. We also began a collaboration with Dr. Martin Spencer who will provide us with purified somatomedin-C and its assay.

Accomplishments

(1) Demonstrated that the infusion of $1,25(\text{OH})_2\text{D}$ into growing rats for 12 days leads to a reduction in the percent of bone that is fully mineralized, as assessed by bone fractionation.

(2) Demonstrated that, although the plasma corticosterone levels of rats during skeletal unloading are no different from controls during the day, these levels are higher in the experimental animals during the night. Thus the circadian rhythm of corticosterone may be altered by skeletal unloading in a way that contributes to the reduction in bone formation in these animals.

(3) Reevaluated the effect of adrenalectomy on bone formation during skeletal unloading by also performing orchiectomy which reduces circulating corticosterone levels to the undetectable range. Under these conditions, a subtle protective effect of the surgical ablations on bone formation was observed during skeletal unloading.

(4) Successfully established primary cultures of chondrocytes, which we plan to use for somatomedin-C studies.

(5) Demonstrated receptors for $1,25(\text{OH})_2\text{D}$ in cultured calvarial bone cells which we hope to manipulate with cytokines to determine whether an interaction between $1,25(\text{OH})_2\text{D}$ and these cytokines can be demonstrated in bone.

Significance of the Accomplishments

Finding #1. In previous studies, we demonstrated that with hind limb unloading, bone formation transiently falls in the unweighted bones of the hind limbs. This is accompanied by a transient fall in serum $1,25(\text{OH})_2\text{D}$ levels, raising the possibility that the fall in $1,25(\text{OH})_2\text{D}$ was instrumental in the fall in bone formation. Now, we have infused $1,25(\text{OH})_2\text{D}$ into rats to prevent the fall in $1,25(\text{OH})_2\text{D}$, but this did not prevent a fall in bone formation. In fact, we seemed to worsen the situation by causing a mineralization defect in the growing bone. We have defined this problem further by analyzing the effect of $1,25(\text{OH})_2\text{D}$ on the distribution of bone among four fractions separated by density (which correlates with degree of mineralization). Our results confirmed our histomorphometric observations that $1,25(\text{OH})_2\text{D}$ infusion inhibits mineralization.

Findings #2 & #3. Our results differed from previous experiments in which only adrenalectomy was performed in that the combination of orchiectomy and adrenalectomy seemed to offer a partial protective effect against the inhibition of bone formation induced by skeletal unloading. This suggests that at least some of the effect of hind limb unloading on bone formation is mediated by corticosterone.

Findings #4 & #5. Our ability to grow bone cells and chondrocytes in vitro provides model systems in which we can test the effect of various cytokines and growth factors on bone growth. We expect that these systems can be applied to cells isolated from bones of growing rats. We plan to determine differences in vitro in the rate of growth of cells isolated from the tibiae of hind limbs of unloaded versus control rats. If so, we can then test the effects of cytokines and growth factors on these cells.

Publications

Bikle, D.D., Halloran, B.P., Cone, C.M., Globus, R.K., and Morey-Holton, E. The Effects of Simulated Weightlessness on Bone Maturation. Endocrinology 120: 678-684, 1987.

Halloran, B.P., Bikle, D.D., Levens, M.J., Castro, M.E., Globus, R.K., and Holton, E. Chronic 1,25-dihydroxyvitamin D₃ Administration in the Rat Reduces the Serum Concentration of 25-Hydroxyvitamin D by Increasing Metabolic Clearance Rate. Journal of Clinical Investigation 78: 622-628, 1986.

Wronski, T.J., Halloran, B.P., Bikle, D.D., Globus, R.K., and Morey-Holton, E.R. Chronic Administration of 1,25-dihydroxyvitamin D₃: Increased Bone but Impaired Mineralization. Endocrinology 119: 2580-2585, 1986.

PHYSIOLOGY OF DEVELOPING GRAVITY RECEPTORS AND OTOLITHOCULAR REFLEXES IN RAT

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Description of Research

This research has the long-term objective of examining the effects of microgravity on the physiology of the adult and developing mammalian vestibular gravity receptors. Our specific aims are as follows: (a) to study the physiological responses of otolith afferents in the adult rat and during postnatal development, and (b) to determine the otolith organ contribution to the rat vertical vestibulo-ocular reflex (VOR).

During the first year, we have made progress in two areas: (a) hardware/software development, and (b) physiological experimentation. First, the proposed studies on single afferents innervating otolith organs require that we obtain large amounts of spike-train data. To automate this process, we have spent considerable time during this year in developing IBM PC-XT based hardware and software routines for data collection/analysis of spike trains and A-D conversion of stimulus signals.

The rat is an extremely important, low-payload mammalian model for vestibular studies, but the orientation of the semicircular canal receptors has not been investigated in this species. Such information is required for proper kinematic referencing of the vestibular output during head movement and is required for analysis of the otolith contribution to the vertical vestibulo-ocular reflexes. Thus, in a second series of physiological experiments, recordings were obtained from primary semicircular canal afferents during "null-point" testing, i.e., during rotation of the animal about different axes to define mathematically each of the canal planes in stereotaxic coordinates. In addition, we have obtained sufficient behavioral data to define semicircular canal position in relation to the freeze-startle position. Findings indicate that the rat, like the rabbit which has already been studied in detail, lowers its head during freeze-startle so as to bring the horizontal canals maximally into the horizontal plane of stimulation.

Accomplishments

The accomplishments during this first year can be divided into two areas:

- (1) Hardware/software development and equipment redesign.
- (2) Physiological studies on vestibular system and head position.

Significance of the Accomplishments

(1) Hardware/software development and equipment redesign:

A certain amount of mechanical and computer development was required during the first year of this grant to ready the recording environment and equipment for the new physiological experiments. Aside from small amounts of time required to prepare new animal head holders, turntable electrode carriages and cabling, the majority of time in this category relates to development of software/hardware routines for spike-train data collection and preliminary analysis using interspike interval histograms.

(2) Physiological studies on vestibular system and head

position: Relatively few vestibular studies have been conducted previously in rat. More importantly, there are no data in this species regarding the best inertial frame of reference for vestibular studies. In other animals, including man, a kinematic system is used which is based upon the stereotaxic coordinate system, and vestibular data such as preferred axes of otolith afferents and/or semicircular canal sensitivity vectors are referenced to this scheme. We have begun our analysis of determining the best vestibular referencing system for the rat in two ways. First, single units innervating the horizontal, anterior and posterior semicircular canals were recorded in anesthetized rats to determine canal position by "null-point" technique. Preliminary data indicate that the three canals are approximately orthogonal, and that the horizontal semicircular canals in rat are inclined 35° (up anterior), relative to the horizontal stereotaxic plane. More data will be required before a planar equation can be derived which describes mathematically each of the semicircular canals in the stereotaxic coordinate system. Once this is done it will be possible to quantitatively evaluate the degree of canal-otolith convergence in central vestibular neurons.

Second, a series of physiological experiments were conducted on pigmented Long Evans rats to evaluate the meaningfulness of the stereotaxic planes in defining vestibular coordinates. In rabbit, a closely related species, Simpson and colleagues have used vestibular coordinates to define the inertial reference system rather than rely upon stereotaxic coordinates. They base their conclusions on the fact that the direction-selective units comprising the visual/optokinetic system are organized in vestibular coordinates, i.e., there are classes of retinal ganglion cells (and neurons within subsequent stages of the

accessory optic system, inferior olive, and cerebellar flocculus) which discharge optimally during visual field motion in the planes of the three semicircular canals. The importance of vestibular coordinates is further acknowledged given that the rabbit normally carries its head, or assumes a startle position, such that the horizontal canals are brought into the horizontal plane.

The rat also exhibits a characteristic freeze-startle position for the head. We initially argued that this position might be used (instead of stereotaxic coordinates) to provide reliable external reference of the vestibular coordinate system. Freeze-startle position was determined in seven rats using a frame-by-frame videotape analysis of head motion. The same animal was tested 10 times to examine intertrial variations. Across the population, the freeze-startle position places the horizontal semicircular canals to within $16 \pm 8^\circ$ (up anterior) of the horizontal plane. Thus, the rat tends to bring the head to an optimal position for stimulating the horizontal canal during the startle response. These data provide a convenient behavioral assay for determining horizontal canal position and may prove extremely helpful during the later studies on the developing labyrinth. However, given the variability of head position between trials and across the population, the freeze-startle head position referencing system was judged to be less reliable than the Horsley-Clarke stereotaxic coordinate system for referencing semicircular canal position.

The numerical descriptions of canal position re. Horsley-Clarke stereotaxic coordinates and the freeze-startle position will permit quantitative studies on the physiology of canal-otolith convergence and the vertical VOR pathways in this species. Further, such knowledge will permit an analysis on the effects of microgravity on the adult and developing mammalian gravity receptors.

STUDIES OF INTERCELLULAR COMMUNICATION AND INTRACELLULAR RESPONSES BY BONE CELLS TO SIMULATED WEIGHTLESSNESS

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Description of Research

The hypogravity of spaceflight and the absence of weight bearing on long bones have many similar effects on the musculoskeletal system. Such effects include loss of muscle and bone mass, a reduction in new bone formation, and decreased bone strength. There may also be a reduction in collagen fiber tensile strength such as found in ligaments, tendons, and soft tissues. Our interest involves studies of events which occur at the cellular level in bone, in an attempt to understand how the absence of weight bearing (a) affects bone cell metabolism, and (b) how the bone cell "senses" or receives information transmitted between the bone matrix and the cell membrane, and between adjacent bone cells.

Due to the lack of flight experiments, the work for this year centered around studies of the non-weight bearing animal model of Dr. E. Morey-Holton and development of new techniques for use on future flight experiments. Two major experiments were:

- (1) Unloading of young rapidly growing rats, to determine the effects of non-weight bearing on the growth and development of the skeletal system.
- (2) Brief unloading experiments (1, 2 and 5 days) in which a tracer protein was injected intravenously at the end of the unloading period and was allowed to circulate for 1 hr. The experiment was ended and tibias and femurs were removed. The tracer can be visualized by light and electron microscopy and can indicate whether the vascular supply of the skeleton has been altered as a result of non-weight bearing. In addition, this tracer (horseradish peroxidase) moves out of the vascular system, with time, and is transported through the osteocyte canalicular system buried within the bone matrix. This effect is dependent on the integrity and "leakiness" of the vessels, which in turn may be influenced by non-weight bearing.

Accomplishments

(1) In rapidly growing rats, a prolonged period of non-weight bearing seems to result in bone cell metabolic activity which is relatively normal.

(a) However, the arrangement of collagen fibrils within the formed bone appears irregular and the architectural form by electron microscopy appears different than normal.

(b) There appears to be more and/or larger vascular

channels within the volume of bone which forms the diaphysis of the long bones. Such an effect could alter the mechanical strength of these bones and perhaps influence their growth and modeling.

(2) In rats injected with tracer to follow the vascular changes, we have only begun our analysis.

(3) The three major bone cell types have now been shown to have specific lectin binding affinities, suggesting that certain carbohydrate groups are associated with specific cell types (see Figure 1).

(a) Osteoclasts (bone-resorbing cells) bind peanut agglutinin lectin at the ruffled border and within cytoplasmic vacuoles.

(b) Osteoblasts (bone-forming cells) show binding affinity in the Golgi complex to tetragonolobus purpureas.

(c) Osteocytes (bone cells within bone matrix) demonstrate concanavalin A binding to their cell membranes and along the canalicular system which connects to adjacent cells.

(4) Using immunocytochemistry on the bone-forming osteoblasts, we are beginning to monitor bone formation by assessment of several morphological criteria:

(a) Golgi activity results in the packaging of procollagen molecules and development of the secretory granules. These granules are quantitated as to their numbers per cell and by their size distribution.

(b) The cytoskeletal system (e.g., microtubules and microfilaments) is responsible for the movement of procollagen secretory granules to the cell membrane for secretion. This cytoskeletal system is being analyzed for quantity and location by immunocytochemistry at the light and electron microscopic level.

(c) The arrangement of bone lamellae and/or the collagen fibril patterns and subsequent mineralization provide an assessment of the mechanical strength of the bone matrix which has already been laid down.

Thus, the events outlined in (a), (b), and (c) form an interlocking series which describe how hypogravity or non-weight bearing affects the skeletal tissues.

Significance of the Accomplishments

Finding #1. Significant force applied to a bone can cause an increase in bone mass and strength, whereas non-weight bearing (absence of normal forces) results in bone loss. However, in rapidly growing animals the genetically driven growth mechanism is active, so that hypogravity or non-weight bearing may affect growth patterns as well as alter the response to normal mechanical stimuli. We are trying, with the use of non-weight bearing growing animals, to sort out these two equally important influences on skeletal development.

Finding #2. The vascular system, which provides nourishment to bone, may also provide an important control over the growth and

LECTIN BINDING TO BONE CELLS

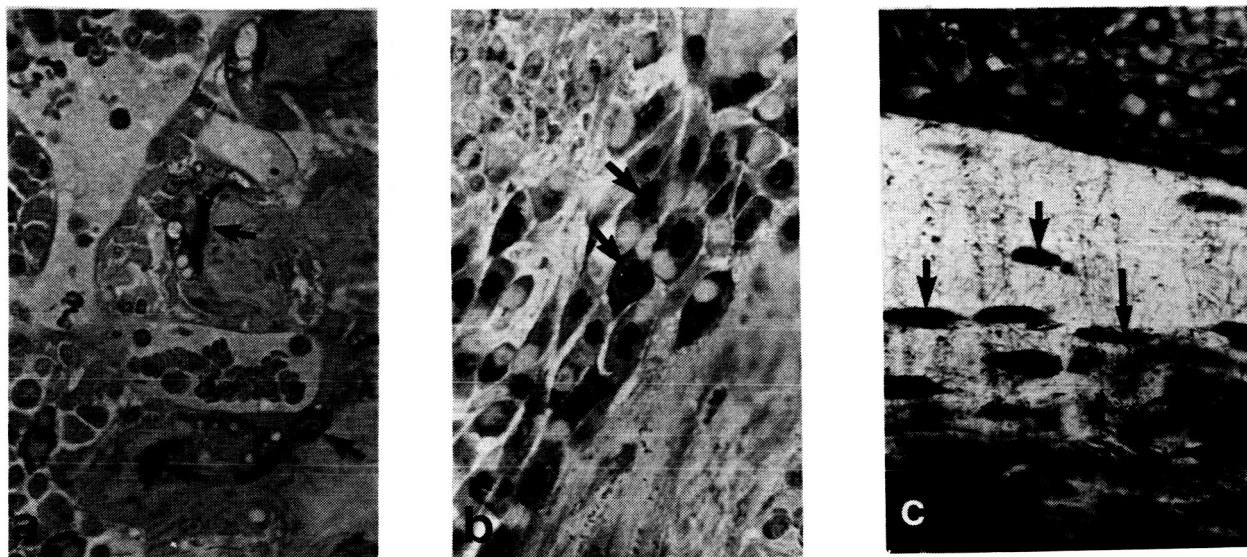


Figure 1. (a) Osteoclasts on surface of bone; arrows indicate lectin binding. (b) Osteoblasts with lectin bound to Golgi complex (arrows). (c) Osteocytes (arrows) covered with lectin which fills lacunae. All magnifications = 525X.

mechanical integrity of the skeleton. Using small molecular weight tracers which are carried through the vascular channels, we can determine whether flow rates are changed as a result of non-weight bearing. This would be a possible cause for many transient changes seen as a result of non-weight bearing.

Finding #3 and #4. Many of the recent activities involve the development of techniques to analyze skeletal function following an experimental period. Most (if not all) spaceflight experiments have to be evaluated following flight, with no opportunity to carry out experimental procedures during the flight period. Thus, interpretation of changes due to hypogravity are made using techniques applied at some significant period of time following the actual flight. This has forced us to develop methods of histochemistry (for certain enzyme localization; or carbohydrate localization by lectin binding studies), and immunocytochemistry, to relate to morphological studies. In addition, the extracellular matrix is a persistent record of events which occurred during the hypogravity period and can be used to help evaluate previous cellular functions.

Publications

Doty, S.B. and Moran, D.J. Ultrastructural Localization of Alkaline Phosphatase in Morphologically Differentiating Neural Crest Cells (Abstract). Journal of Cell Biology 101(Pt. 2): 122, 1987.

Wronski, T.J., Morey-Holton, E.R., Doty, S.B., Maese, A.C., and Walsh, C.C. Histomorphometric Analysis of Rat Skeleton Following Spaceflight. American Journal of Physiology 252: R252-255, 1987.

GROWTH AND DIFFERENTIATION OF MAMMALIAN TISSUES EXPOSED TO HYPERGRAVITY IN VIVO AND IN VITRO

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Description of Research

A basic question of the Space Biology Program is how gravitational changes affect developing systems. Effects of gravitational changes on post-implantation mammalian development can be studied on Earth only by using excess gravity produced by centrifugation--a system routinely used in the Soviet Union for predictions of microgravity effects. This laboratory has been studying the effects of altered gravity on development of the mammalian skeletal system, which is known to be responsive to gravitational changes in vivo and in vitro. Mice were chosen for this study because their small size makes them less susceptible to hypergravity, thus lessening any effects on development due to effects on the mother. Also, the mouse limb bud is a well-characterized system which can be maintained in vitro.

For in vivo studies, four week-old male and female mice were placed on a small animal centrifuge and allowed to adapt to the excess g force (2.3-3.5 g). Mice were weighed every week to assess their growth, and estrus studies were used to determine the appropriate time for pairing of males and females. In order to assess skeletal development, 18-day fetuses were stained with alizarin red, and ossified areas of the long bone were measured using computerized planimetry.

In one study, mice were sacrificed after a year at excess gravity, and their long bones and skulls were subjected to a comprehensive morphometric analysis to determine if any changes in size and shape had occurred due to the increased loading.

Accomplishments

(1) Mice centrifuged for one year at 2.3-3.5 g weighed significantly less than 1 g controls.

(2) Crown rump lengths (CRLs) of 18-day fetuses were significantly greater than those of controls, in contrast to previous studies.

(3) Ossified areas of long bones of these fetuses were found by morphometric analysis to not differ significantly from long bones of control fetuses in most instances (exceptions were increases in the widths of right femurs and lengths of left fibulae).

(4) As in previous studies, almost all the fetuses were female.

(5) In a comprehensive morphometric analysis of post-natal development of bones of centrifuged animals, significant differences in size and shape of long bones were noted, illustrating changes in bone architecture in response to loading.

(6) Cephalometric analyses (the first such study on centrifuged animals) showed that skulls of centrifuged animals are elongated and compressed.

(7) In our fourth excess g study, using an improved centrifuge and a high-fat content chow, weights of centrifuged animals were again lower than weights of controls.

(8) Estrus studies have been expanded, and have aided in increasing the number of pregnancies resulting from timed matings, although there are still fewer pregnancies in centrifuged animals.

Significance of the Accomplishments

The significance of Finding #1 is not only the demonstration (once again) of the effect of gravitational changes on mice, but also that a small inexpensive animal centrifuge can produce results identical to those obtained using large, very expensive centrifuges. The use of small centrifuges allows an increase in the number of scientists, institutions, and species involved, and the types of studies done.

Findings #2 and #3 demonstrate the importance of maternal age in centrifugation studies of mammalian development--a factor that is not always taken into consideration.

The effect of exposure to excess g on sex ratios is seldom addressed in gravitational studies. Although skewed sex ratios may result from stress, that is not likely in this case (Finding #4) because the animals were adapted to excess g before the study began. This result is similar to the finding by Little et al. that pilots who pull excess g forces have more female children than those who do not, and indicates that the effect of excess g on sex determination is an area requiring additional studies.

In studies of effects of gravitational changes on bones, Wolff's law has been interpreted as meaning that calcium content should increase in excess g and decrease in microgravity. The demonstration of significant morphometric changes in bones of centrifuged mice (Finding #5) emphasizes that more attention should be paid to the changes in architecture predicted by Wolff's law.

Finding #6 demonstrates that, even in mice, nonweight-bearing bones are affected by gravitational changes.

MECHANOSENSORY-ACTIVATED AND VOLTAGE-DEPENDENT ION CHANNELS IN STATOCYST HAIR CELLS OF HERMISSENDA CRASSICORNIS

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Description of Research

The neural processing of gravitational-produced sensory stimulation of statocyst hair cells in the nudibranch mollusc Hermisenda has been studied. Our goal in these studies has been to show (a) how gravireceptor neurons "sense" or transduce gravitational forces, (b) how gravitational stimulation is integrated so as to produce a graded receptor potential, and ultimately the generation of an action potential, and (c) how various neural adaptation phenomena which hair cells exhibit arise. Our approach to these problems has been primarily electrophysiological.

Previous research has shown that motile cilia on statocyst hair cells transduce gravitational forces, through active mechanical interaction with statoconia suspended inside the statocyst. The mechanical interaction of the hair cell cilia and the statoconia results in a mechanical deformation of the cilia and hair cell membrane, and the opening of ion channels. These ion channel openings result in an influx of cations into the cell and a resulting depolarization.

During the past year, we have studied these mechanosensory-activated ion channels through the use of fluctuation analysis of current "noise" observed under voltage-clamp. We have also studied several other classes of ionic channels in the hair cells using voltage- and single-channel patch clamp techniques. These channels (primarily K⁺ selective) subserve the hair cells' integrative response to gravitational stimulation. They are activated by changes in the membrane potential of the cell, rather than by mechanosensory stimulation per se.

Accomplishments

(1) Previous intracellular recording studies of Hermisenda hair cells under current-clamp conditions by other investigators have presented evidence that the increased voltage noise and depolarizing generator potential produced by mechanical- or gravitational-induced interaction between hair cell cilia and statoconia reflects mechanosensory activation of a Na⁺-selective channel in the receptor membrane. We have studied these channels by means of fluctuation analysis of ionic current noise, under voltage-clamp control. Current noise amplitude is typically 1-2 orders of magnitude greater for hair cells exposed to 1 g force

ELECTRICAL CURRENT NOISE RECORDED IN MOLLUSC STATOCYST HAIR CELLS

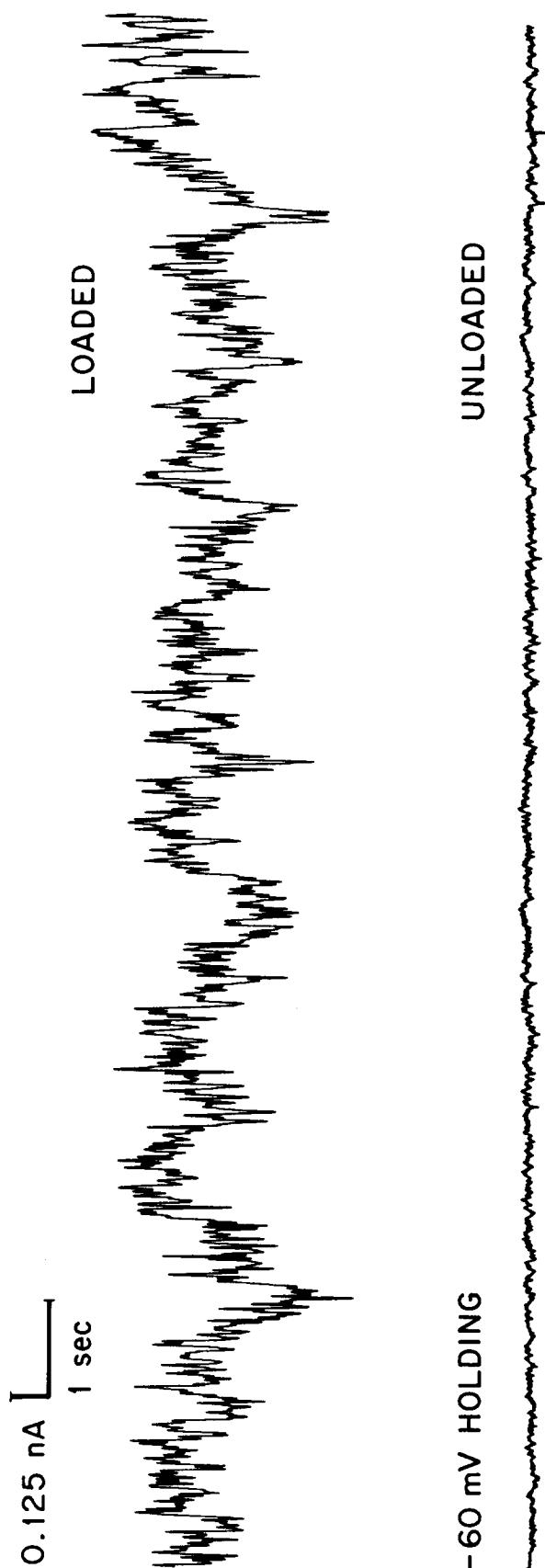


Figure 1. Current noise from Hermisenda statocyst hair cell in "loaded" vs. "unloaded" conditions. Cell was voltage-clamped at -60 mV in a standard ASW solution, using conventional two microelectrode voltage-clamp techniques. Tilting of preparation resulted in the mass of statocyst coming to rest on or near the caudal portion of the statocyst (loaded condition). Tilting 90° in the opposite pole direction resulted in the statocyst moving towards the opposite pole of the cyst, away from the caudal hair cells (unloaded condition). Note the two conspicuously large bursts of inward (down) current in the loaded condition. These are likely to reflect the simultaneous interaction of a statocyst with one or more motile cilia.

by tilting ("loaded" condition, Figure 1) than in the non-stimulated ("unloaded") case. Current noise amplitude increases in a potential linear fashion as the steady state holding potential of the hair cell is made more negative than -40 mV. Removal of extracellular sodium results in a reversible decrease in noise amplitude, though it does not completely abolish it. In addition, the power spectra for many (approximately 50%) cells was non-regular with a peak in the 7-12 Hz range. This coincides with the mean frequency of the inherent motions of statocyst hair cells. Individual records of current noise revealed that the 7-10 Hz peak in the power spectra corresponded to the presence of large, discrete, inward currents with an extrapolated reversal potential $> +10$ mV. Collectively, these results indicate that Na^+ ions make a significant contribution to the ionic current through the mechanosensory-activated channels.

(2) Three distinct components of voltage-dependent outward (K^+) current were observed under voltage-clamp. Two of these appear to be identical to K^+ currents previously described in detail in a variety of central neurons in other molluscs. When depolarized from a holding potential of -60 mV to potentials more positive than -40 mV, a rapid (approximately 10 ms to peak at 0 mV), transient, 4-aminopyridine (4-AP) sensitive, outward current was observed I_A . The voltage-dependent inactivation of I_A was studied using a twin-pulse protocol in which the cell was first held at -60 mV, then stepped to a variable potential (from -100 to 0 mV), before being stepped to a final potential of 0 mV. I_A was half-inactivated at -45 mV, which is considerably more positive than values typically reported in other cells and implies that I_A can contribute to the resting membrane conductance of the hair cell. Activation of I_A was also studied with a twin-pulse protocol. The cell was first stepped to -100 mV, to remove resting inactivation, and then to a variable potential from -60 mV to 0 mV. Activation was steeply voltage-dependent at potentials more positive than -40 mV.

(3) A second slower component of outward current, which showed a moderate rate of inactivation, was observed when hair cells were depolarized to potentials more positive than 0 mV. At +20 mV, time to peak for this current was 40-60 ms. This current was blocked by 100 mM TEA and appears to be similar to the "delayed rectifier" current (I_K) seen in other molluscan neurons and Hermisenda Type B photoreceptors.

(4) With I_A and I_K blocked by 5 mM 4-AP and 100 mM TEA, an extremely slow outward current was elicited by depolarization to potentials > -30 mV. This current, which showed little inactivation during depolarizing steps as long as 15 sec, increased in amplitude during a sustained depolarization, reaching its steady-state value after approximately 5 sec. Although the kinetics of activation appear similar to those of a calcium-activated potassium current (I_{K-Ca}) in Hermisenda Type B cells, this current proved to be insensitive to: (a) changes in extracellular Ca^{2+} concentration over the range of 0-100 mM, (b) addition of calcium channel blockers to the bath (Cd^{2+} , 5 mM), and (c) intracellular injections of EGTA, a calcium chelator.

However, each of these manipulations produced the expected changes in the magnitude of a transient, inward, voltage-dependent (calcium) current. We conclude that the delayed outward current is not a calcium-activated current, although it is most likely a potassium current, since: (a) both peak (I_A) and steady-state (measured 500 ms following depolarization) outward current components show the same reversal potential in elevated K^+ solutions (-12 mV in 300 mM K^+). I_A is extremely K^+ selective and the similar reversal potential for I_A and the delayed component implies a similar K^+ selectivity. (b) An outward tail current was observed in 4-AP and TEA when the cell was stepped back to -60 mV from command steps more positive than -30 mV, consistent with our estimate of E_K in 10 mM K^+ ASW (approx. -100 mV).

(5) Single-channel K^+ currents were recorded from Hermisenda hair cells using the cell-attached patch-clamp recording configuration. Open time distributions indicate that no single exponential decay function will suffice. This implies the presence of multiple open states. The channel is clearly voltage-dependent and is open a large fraction of the time at potentials more positive than rest (approximately -50 mV). This channel may underlie one of the slower components of macroscopic K^+ current seen in the previous voltage-clamp studies.

Significance of the Accomplishments

Finding #1, that the current noise of hair cells increases during gravitational stimulation and that the channels are selectively permeable to Na^+ ions, represents the first direct information concerning the biophysical properties of mechanosensory-activated ion channels in an invertebrate neuron specialized for gravireception.

Finding #2-#4, that there are at least three kinetically and pharmacologically distinct components of the net outward current elicited by depolarization, represents the first direct analysis of integrative ion currents in an invertebrate gravireceptor neuron.

Finding #5, our patch-clamp recordings of single, voltage-dependent K^+ channels in Hermisenda hair cells, represents the first direct single-channel recordings from any gravireceptor neuron.

Collectively, our findings provide a detailed and comprehensive view of those ionic channels that subserve the transduction and integration of gravitational stimulation in a primary gravireceptor neuron under normal gravitational conditions.

Publications

Farley, J. Contingency-learning and Causal Detection in Hermisenda: I. Behavior. Behavioral Neuroscience 101: 13-27, 1987.

Farley, J. Contingency-learning and Causal Detection in Hermisenda: II. Cellular Mechanisms. Behavioral Neuroscience 101: 28-56, 1987.

Farley, J. Single Ca^{2+} -Dependent K^{+} Channels from Hermisenda B Photoreceptors (Abstract). Society for Neuroscience Abstracts 13: 559, 1986.

Farley, J. and Alkon, D. Cellular Analysis of Gastropod Learning. In: Cell Receptors and Cell Communication in Invertebrates (ed. by A.H. Greenberg). Basel: Karger, pp. 220-226, 1987.

Farley, J. and Auerbach, S. Protein Kinase C Activation Induces Conductance Changes in Hermisenda Photoreceptors Like Those Seen in Associative Learning. Nature 319: 220-223, 1986.

Farley, J. and Rudy, B. Single K^{+} Channels from Rat and Hermisenda Brain Incorporated into Lipid Bilayers on Patch-clamp Pipettes (Abstract). Biophysical Journal Abstracts 49: 575a, 1986.

Fisher, D. and Farley, J. Imipramine Inhibits PKC-induced Electrophysiological Changes in Hermisenda B Photoreceptors (Abstract). Society for Neuroscience Abstracts 13: 994, 1986.

Grover, L. and Farley, J. Serotonin Mediates in vitro Conditioning Effects on Type B Photoreceptors in Hermisenda (Abstract). Society for Neuroscience Abstracts 13: 1338, 1986.

Lipkin, S., Farley, J., and Rudy, B. Protein Kinase C Effects on Single K^{+} Channels from Mammalian Brain (Abstract). Society for Neuroscience Abstracts 13: 1343, 1986.

Reeves, R., Farley, J., and Rudy, B. c-AMP Dependent Protein Kinase Opens Several K^{+} Channels from Mammalian Brain (Abstract). Society for Neuroscience Abstracts 13: 1343, 1986.

Schuman, E.M., Farley, J., and Richards, W.G. Neural Substrates of Locomotion in Hermisenda (Abstract). Society for Neuroscience Abstracts 13: 1205, 1986.

Wu, R., Grover, L., and Farley, J. Pairing- and Stimulus-Specific Food Aversions in Hermisenda (Abstract). Society for Neuroscience Abstracts 13: 862, 1986.

OTOCONIA CALCIFICATION PROCESS: A CHICK EMBRYO MODEL

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Description of Research

The objectives of this study are: (a) to establish the genesis of statoconial units (gravity sensing structures), (b) to analyze the subcellular arrangements of the subunits making up statoconia units, and (c) to correlate biochemical properties of statoconia constituents with their ultrastructural appearance.

Answering these and other questions posed below will help to establish ground-based data for further evaluating changes that gravity has produced in statoconia. For instance, it is known that statoconial units may be born not fully calcified. However, it is not known how the final process of calcification takes place.

While many hypotheses have been enunciated attempting to explain how calcification occurs, conclusive proof does not exist. Since each sensory system undergoes critical periods of development in which modification, refinement, and/or establishment of adult characteristics set in an important aspect of the puzzle, science must solve the problems created by modified gravity during space exploration and colonization.

Accomplishments

The developing statoconia of chick embryos were examined with the light and electron microscopes. Biochemical and histochemical properties of these gravity-related structures were studied. In the chick, as in mammals, statoconia are formed by an organic matrix and by calcite as calcium carbonate. Mineralization of the statoconia occurs gradually and it seems to undergo periods in which the process is accelerated. Critical periods probably occur during narrow stages of development because inhibition of statoconia formation by carbonic anhydrase inhibition is more pronounced when embryos are injected prior to final mineralization of individual statoconia. Ongoing experiments have shown that incorporation of the isotope calcium 45 is higher in 11 than 7 day-old embryos, probably because statoconial units are calcified gradually.

Cationic and anionic stains bind to different chemical groups. Ruthenium red, alcian blue, and tannic acid are among these stains. Each binds selectively to different moieties of glycosaminoglycans. Different areas of the chick statoconia are stained with these substances indicating possible variations in

the biochemical properties of the organic matrix. The properties described in the legends for Figures 1-4 clearly indicate that at least three different moieties exist in the matrix, as judged by their affinity to different cationic stains.

Biochemically, (e.g., electrophoretic separation) as many histochemical patterns are obtained, these different histochemical moieties, if associated with proteins, should yield at least as many protein bands.

These preliminary findings indicate that the uncalcified otolithic membrane may contain calcium binding proteins, which at some time in development unlock the key to the receptors (if present) responsible for the calcification process of statoconia. In this respect, immunohistochemical and immunoblotting techniques are incorporated into this research to properly define the nature of the protein bands.

Gel electrophoresis analysis of young statoconial masses yielded several protein bands that seem to be unrelated to blood and/or proteins from macular tissue. Answers to questions addressed in the last application and now in the objectives stated below are important and the results obtained may be applicable to research in other calcifying tissues such as bone and teeth. Some of the findings may help in the interpretation of bone density loss after prolonged spaceflights since normal developmental processes leading to calcification may be related to metabolic pathways of disorders produced by modified gravity. Ultrastructural analysis is very important to determine among other things: (a) how macromolecules are embedded or entrapped within the matrix; (b) properties and orientation of subunits making up the crystals; (c) mechanisms by which the matrix contributes to regular crystal growth.

Significance of the Accomplishments

The results described above emphasize the necessity to pursue this type of research since our knowledge about the effect of microgravity on statoconia is limited and relatively non-existent for embryonic vertebrates.

There is evidence that statoconial units undergo drastic morphological modifications, which must have underlying biochemical mechanisms. Thus, the data described above allow the investigators to ask the questions whose answers will help characterize the components of the organic matrix.

Several of those questions are listed below:

- (a) Study changes in the stainability of various areas of statoconia by different binding cationic dyes.
- (b) Determine critical period for mineralizations of chick statoconia by examining deposition of calcium 45 at various stages prior to final calcification.
- (c) Study selectivity of the organic components for various

Figure 1. The organic (arrowheads) and inorganic (arrows) components of statoconia are shown. Tissue was histochemically stained with osmic potassium pyroantimonate, which has a preferential affinity for calcium (arrows). TEM X50,000. 15 days of incubation.

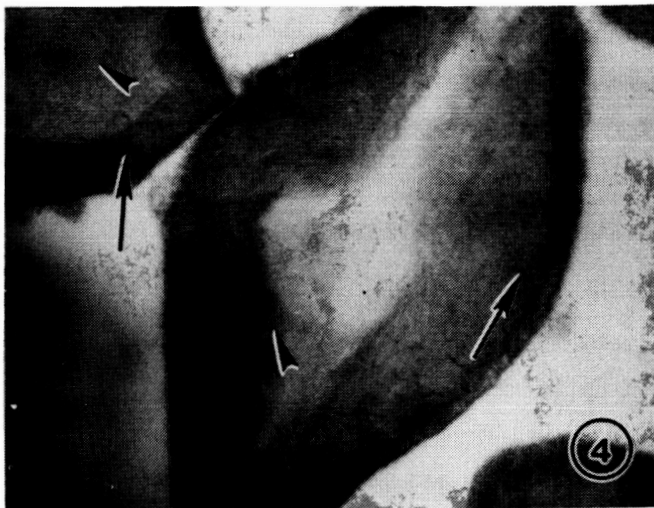
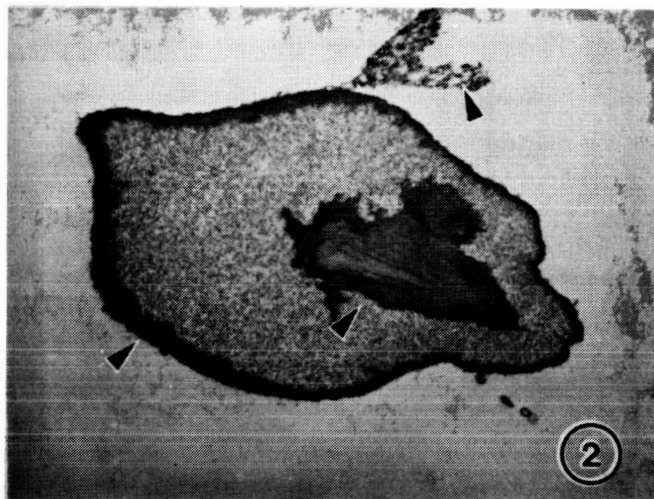
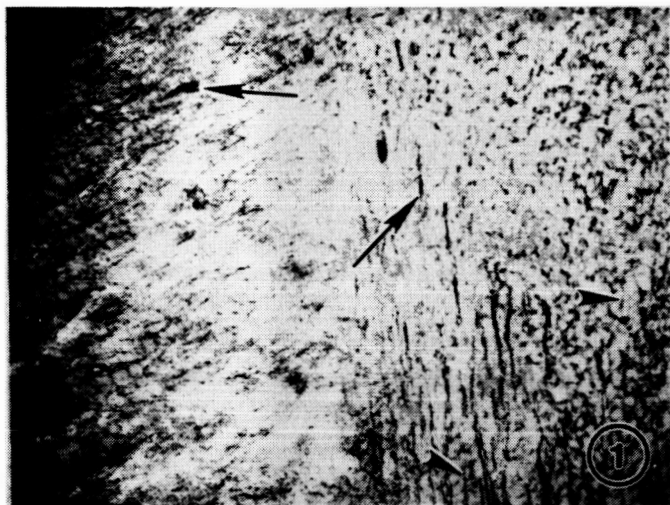
Figure 2. Tannic acid is a polyphenolic compound that prevents extraction of labile substances during EM preparations, and joins glycoproteins regardless of their electrical charges. Tannic acid can therefore prevent loss of substances that are otherwise extracted from tissues fixed with aldehydes alone. This micrograph taken from an unstained section illustrates possible loci of glycoprotein conjugate (electron-dense) in otoconia (arrowheads). TEM X8,300. 1 day post-hatch.

Figure 3. Ruthenium red stain is a cationic compound which has been found to protect protoglycan macromolecules (large, up to 35 nm in diameter) against enzymatic degradation. In the presence of osmic acid, ruthenium red reacts with glycosaminoglycans. The matrix between (asterisks) and not within statoconia (o) reacted with ruthenium red (arrows). TEM X10,000. Unstained, 1 day post-hatch.

Figure 4. Alcian blue is a cationic compound (dye) that reacts with hyaluronic acid (a small size molecule glycosaminoglycan less than 3 nm in diameter). In this unstained section, hyaluronic acid seems to be present throughout the statoconia and it seems to consist of large (arrows) and small fibrillar (arrowheads) subunits. TEM X10,000. 1 day post-hatch.

ORIGINAL PAGE IS
OF POOR QUALITY

PROPERTIES OF CHICK STATOCONIA ORGANIC MATRIX



commercially available antibodies known to react with glycolyx, which is ultrastructurally similar to the unmineralized organic matrix.

(d) Analyze statoconia at several stages biochemically (e.g., two-dimensional gel electrophoresis) to see if bands obtained to date change with developing time and with different calcifying states, and also to determine specificity and reliability of the bands obtained thus far.

(e) X-ray microanalyze cryosections obtained from 6-day and 10-day-old embryos to investigate whether there are significant changes in the depositions of the inorganic matrix with time.

Publications

Cohen, G.M. and Fermin, C.D. Development of the Embryonic Chick's Tectorial Membrane. Hearing Research 13: 29-39, 1985.

Fermin, C.D. and Igarashi, M. Review of Statoconia Formation in Birds and Original Research in Chicks (Gallus domesticus). Scanning Electron Microscopy 4: 1649-1665, 1986.

Yoshihara, T., Usami, S., Igarashi, M., and Fermin, C.D. Ultracytochemical Study of Ouabain-sensitive, Potassium-dependent p-Nitrophenylphosphatase Activity in the Inner Ear of Squirrel Monkey. Acta Otolaryngology 103: 161-169, 1987.

HOMEOSTASIS IN PRIMATES IN HYPERACCELERATION FIELDS

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Description of Research

The ultimate goal of this research program is to understand the role of gravity in influencing the physiology of living organisms. Of particular interest are the physiological mechanisms leading to adaptation of an organism to an altered gravitational environment. Included in these responses are identification of receptors, pathways of information transfer (neural and endocrine), mechanisms of integration of information, and pathways and mechanisms affecting the organism's response to alterations in gravity.

The adaptation of homeostatic systems to changes in gravitational loading are poorly understood. Such changes in centrifuged animals include depressed body temperature, alterations in the circadian timekeeping system, and changes in the level of arousal. To date, research interests in this laboratory have focused on the sensitivity of these and other homeostatic systems to alterations in gravity. This research has provided both a demonstration of these system's responsiveness to gravity as well as efforts to elucidate the underlying mechanisms of the responses. Further, this program has focused on the responses of the whole organism (primates and rodents) to further understand the interaction between the various physiological systems of interest. This research has required the ability to alter the dynamic environment of the organism. At the Chronic Acceleration Research Unit at the University of California at Davis, four centrifuges (8-18 ft diameter) are available. These facilities provide acute and chronic g fields ranging from 1 to 20 g. The research accomplished in the past year has examined the responses of primates to altered fields (2 g) for up to 10 days exposure duration. Additionally, control studies at 1 g have begun to examine the neural control mechanisms integrating the circadian timekeeping system with those of body temperature and sleep-wake cycles. Finally, an opportunity to examine both rodents and primates in the microgravity environment was utilized with the advent of Spacelab(SL)-3.

Accomplishments

- (1) The presence of a hyperdynamic environment depresses rodent body temperature for an extended period (more than 20 days).
- (2) Exposure to prolonged (60 days) 2 g field leads to a

reduction on the average amplitude of the circadian temperature rhythm which persists for up to 20 days prior to the animal's recovery to baseline.

(3) Preliminary data suggest that while the body temperature rhythm is depressed, there is a change in the normal entrainment by the 24-hr light-dark cycle.

(4) Rats centrifuged at 2 g for 7 days demonstrated a decrease in the hippocampal serotonin receptor level. This is the opposite to that found in rats exposed to zero g for 7 days on SL-3.

(5) The metabolic development of the suprachiasmatic nucleus (SCN), a critical locus for circadian function, was examined in 1 g control rats and rats born and raised in a 2 g field via centrifugation. During normal development, the SCN differentiates into high metabolic and low metabolic portions. Low metabolic regions first form at the dorsal edge by 28 days of age. This continues to mature until the dorsal half and the posterior portion of the SCN exhibit decreased metabolism. The development of 2 g experimental rats differs from 1 g controls in that the low metabolic regions first form at the posterior edge of the SCN by 31 days of age. Low metabolic regions continue to expand to the dorsal half of the SCN.

Significance of the Accomplishments

In general, findings #1-#3 demonstrate the response of mammals to increased levels of gravitational loading. The response underscores the significant delay (i.e., days) in the adaptation of these organisms to changes in the dynamic environment. For example, finding #1 demonstrates that body temperature is depressed for an extended period of time as a result of exposure to hyperdynamic environment. Similarly, exposure to hyperdynamic fields also leads to multiple-day recovery periods for the temperature rhythms of these animals. Finding #3 also demonstrates an apparent change of the utilization of photic information by animals in a microgravity environment. That rodent body temperature rhythms may not synchronize to the 24-hr light/dark cycle is a novel finding. Historically, such observations have only been made in primates and rodents exposed to the microgravity environment. That such a change in photic response only occurs in the temperature rhythm further underscores our understanding of the circadian timekeeping system and photic entrainment.

Finding #3 provides us with continuing information regarding the underlying mechanisms of the organization of these various physiological systems. That the suprachiasmatic nucleus plays a major role in the regulation of the circadian timekeeping system in rodents is crucial to our understanding of gravitational influences. With such information, we hope to begin to define how control systems perceive information regarding changes in gravity and how they modify the regulated levels to new steady states.

Finding #4 demonstrates both the repeatability of the observation that gravity level influences the regulated level of serotonin receptors in the hippocampus of the central nervous system and that the response is of the opposite sign in the hyperdynamic environment as compared to the microgravity environment.

Finding #5 demonstrates that rats born and raised in a 2 g field exhibit patterns of SCN development that are different than 1 g controls. This suggests that the 2 g environment may affect the normal development of circadian rhythms.

NEURAL MECHANISMS BY WHICH GRAVITATIONAL STIMULI AND STRESS AFFECT THE SECRETION OF RENIN AND OTHER HORMONES

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Description of Research

The long-term goal of this research is delineation of the neural pathways and transmitters that mediate changes in the secretion of renin and other hormones concerned with regulation of salt and water balance in response to gravitational and other stimuli. Evidence from this laboratory indicates that stimulation of serotonergic neurons on the dorsal raphe nucleus of the midbrain increases renin secretion, and that these neurons project to the mediobasal hypothalamus (Kartesz, et al., Neuroendocrinology 34: 323-326, 1982). The initial goal of determining how the message got from the hypothalamus to the renin-secreting cells in the kidney was accomplished by pharmacological experiments demonstrating that the pathway was sympathetic. The present goal is to determine by the production of discrete lesions the parts of the hypothalamus and brainstem that are involved in serotonin-mediated increases in renin secretion. In addition, we want to determine the role of the brain in the renin responses to other stimuli. Therefore, we have developed and standardized a variety of stimuli which act in different ways to increase renin secretion. These include: (a) administration of the serotonin-releasing drug p-chloroamphetamine (PCA); (b) the psychological stress of immobilization; (c) the postural stress of 45° head-up tilt; (d) the volume depletion stress of a low sodium diet; and (e) the acute volume stress of nonhypotensive hemorrhage.

Accomplishments

(1) Like the PCA response, the renin responses to immobilization and head-up tilt were shown to be blocked by the beta-adrenergic blocking drug propranolol, indicating that the final common pathway from the spinal cord to the renin-secreting cells in the kidney is sympathetic in all three situations.

(2) Although the increase in renin secretion produced by PCA was abolished by lesions of the dorsal raphe nucleus, the response to immobilization, head-up tilt, and a low sodium diet was not. Therefore, these latter stimuli do not act by increasing the discharge of the serotonergic neurons in the dorsal raphe nucleus.

(3) Bilateral lesions of the paraventricular nuclei reduced or abolished the increase in plasma renin activity (PRA) produced by PCA, immobilization, head-up tilt, and a low sodium diet. However, paraventricular lesions also produced a marked, reproducible decline in the plasma concentration of renin

substrate. Since substrate is rate limiting for the generation of angiotensin I in plasma, we remeasured generation of angiotensin I after adding exogenous renin substrate, thus measuring plasma renin concentration (PRC). Like the PRA response, the PRC response to PCA was also abolished by paraventricular lesions. However, the PRC response to immobilization, head-up tilt, and a low sodium diet was normal. Consequently, it appears that the renin-stimulating serotonergic neurons in the dorsal raphe nucleus project to the paraventricular nuclei, but that immobilization, head-up tilt, and a low sodium diet do not act via paraventricular nuclei.

(4) The PRA response to nonhypotensive hemorrhage was normal in rats with bilateral paraventricular lesions, but the PRC response was much greater than normal. Thus, it appears that the body compensates for the deficient production of angiotensin I (and, consequently, of angiotensin II) by producing a stronger stimulation of renin secretion with this particular stress when there are lesions of the paraventricular nuclei.

(5) Bilateral lesions of the ventromedial nuclei abolished the PRA response to PCA, immobilization, head-up tilt, and a low sodium diet without producing any change in plasma substrate concentration. Thus, it appears that the ventromedial nuclei are nodal points in the neural pathways responsible for the increase in renin secretion produced by a variety of different stimuli. We will soon embark on experiments to determine whether these nuclei mediate responses to multiple stimuli or exert some sort of overall tonic effect on the responsiveness of the juxtaglomerular cells.

(6) Confirming others, we found that paraventricular lesions block the ACTH response to stress but do not decrease resting plasma ACTH. Since adrenal glucocorticoids, estrogens, and thyroid hormones all stimulate the secretion of renin substrate by the liver, it seems likely that the effects of paraventricular lesions on substrate are due to endocrine changes. The paraventricular nuclei are involved in the regulation of TSH as well as ACTH secretion, and hypophysectomy has been shown by us to produce a decrease in renin substrate. In preliminary experiments, the time course of the decline of substrate produced by paraventricular lesions paralleled that produced by hypophysectomy. However, this experiment needs to be confirmed, expanded, and supplemented with hormone replacement experiments. This will be an important goal of the research during the coming year.

(7) To see if vasopressin-secreting efferents from the hypothalamus to the cardiovascular regulatory areas in the brainstem and spinal cord are involved in the regulation of renin secretion, the renin secretory responses to the stimuli we have been employing were tested in Brattleboro rats that were unable to produce vasopressin in their brains. Responses to all the stresses were greater than normal. Conversely, intraventricular injection of vasopressin appeared in very preliminary experiments to inhibit renin secretion, and intraventricular administration of an oxytocin agonist appeared to stimulate renin secretion. Further experiments of this type are planned.

(8) Blood pressure, heart rate, and vasopressin responses to head-up tilt have been measured in addition to renin during the past year. There were no differences in blood pressure and heart rate between rats with lesions of the paraventricular nuclei and controls. In very preliminary experiments with vasopressin and renin antagonists, it appeared that it was primarily the renin-angiotensin system rather than vasopressin that maintained blood pressure during head-up tilt.

Significance of the Accomplishments

The experiments with PCA have now demonstrated that there is a serotonergic pathway which projects from the dorsal raphe nuclei to the paraventricular nuclei and the ventromedial nuclei of the hypothalamus; that the projection from the paraventricular nuclei to the brainstem and spinal cord may be oxytocinergic, although this point needs additional research; and that the pathway from the spinal cord to the renin-secreting cells is sympathetic. The demonstration that paraventricular lesions lower circulating renin substrate is important because it raises the possibility that substrate secretion is under neural control, either via the pituitary or by direct neural pathways. If the endocrine pathways prove to be key, this once again demonstrates the importance of neuroendocrine mechanisms in the maintenance of cardiovascular homeostasis. The discovery that lesions of the ventromedial nuclei appear to abolish the increase in renin secretion produced by many different stimuli, without affecting the concentration of renin substrate in the plasma, makes the position of the hypothalamus in the regulation of fluid and electrolyte balance more prominent than previously suspected.

Publications

Alper, R.H., Deschepper, C.F., and Ganong, W.F. Effect of Hypophysectomy on Dipsogenic Stimuli: Evidence for Angiotensin Supersensitivity. American Journal of Physiology 251: R53-R58, 1986.

Ganong, W.F., Gotoh, E., Murakami, K., and Steele, M.K. Neuropeptides, Cardiovascular Homeostasis, and Hypertension. Japanese Journal of Hypertension 8: 55-70, 1986.

Gotoh, E., Murakami, K., Golin, R., and Ganong, W.F. Hypothalamic Regulation of Plasma Renin Activity (Abstract). Abstracts of the 1st International Congress of Neuroendocrinology, p. 86, 1986.

EFFECTS OF HYPOGRAVITY ON SYNAPTOGENESIS IN CELL CULTURE

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Description of Research

Safe habitation of outer space requires that embryonic development occur normally. This period of maturation, in the life cycle of any organism, is the most sensitive to environmental perturbation even in its native, terrestrial environment. It is therefore prudent to anticipate that the pronounced reduction of the only constant environmental variable in space--gravity--is likely to affect organismic development to maturity.

The aim of this research is to examine the development of embryonic cells which play a crucial role in the survival of the organism: nerve and muscle cells. These cells are particularly vulnerable to environmental perturbation since they undergo final mitotic division early in the embryo. Alterations in normal development are likely to have permanent effects on brain and muscle function of the organism should it survive such alterations.

Although it is presently technically difficult to examine in detail, generational development of even short-lived organisms under actual microgravity, simulation methods provide a sound alternative for examining the specific cellular mechanisms which may be affected during development in space. The clinostat has proven to be a reasonably faithful simulator of microgravity, especially for small organisms and cells.

In the earlier phases of this work, the following objectives were achieved: (a) design and fabrication of a clinostat to carry nerve and muscle cells grown in culture; (b) verification of cell survival and viability under clinorotation at speeds of 1-100 rpm; (c) observations of clinorotation-induced changes in cell morphology; and (d) development of a unifying working hypothesis, based on present findings, and an extension of this hypothesis for further testing of predictions derived from it.

Accomplishments

During the past year, we obtained statistically significant data which demonstrate that clinorotation of cultured nerve and muscle cells is associated with morphologic changes and functional alterations, expressed in altered cell morphology which may be taken as evidence that clinorotation affects cell metabolism, synthetic capacity, and intracellular functions.

Since clinorotation is generally accepted to be a faithful simulator of microgravitational conditions, then the inevitable implication arises that subjecting cells to microgravity during crucial periods of early development may result in adverse alterations in cell organization and tissue function. This is especially true for highly differentiated cells like nerve and muscle, which go through their final mitotic division early in development and in which alterations during the post-mitotic period may therefore propagate through the adult stage of the organism. It is prudent, then, to continue studying the model. The ultimate objective will be to define the mechanisms whereby these alterations are produced and therefore to be in a position of neutralizing these adverse effects during prolonged exposure to this novel environment.

Figure 1 shows (top row from left to right) a pictorial summary of the effects of clinorotation at 1 and 10 rpm on muscle and nerve cell morphology. The myocyte surface area is increased (compare control to 1 rpm), the nucleus enlarges, the nucleoli become more prominent, and the amount of yolk remaining 48 hr after seeding the cells in culture is larger in rotated cells. Another feature illustrated in this row is the presence of large, irregular, and frequent varicosities which appear like aneurysms along the shaft of the neurites (see especially top row, left column). We have studied these alterations in a very large number of cultures in order to verify that these remarkable changes were not a chance occurrence.

It was further found that simulation of microgravity decreases the ability of clinorotated cells to respond to trophic factors found in Xenopus Embryo Extract (XEE) and to laminin, another trophic factor. These supplements have been shown to increase neurite production cell viability. In general, it is considered that XEE supplements the cultures in much the same way as chick embryo extract (or fetal calf serum) serves to supplement other culture systems with, to date, undefined but partly obligatory factors. The micrographs in the next three rows of Figure 1 show typical cells from cultures grown in increasing concentrations of XEE (as indicated). The results show that, in general, addition of XEE did not obliterate the effects of clinorotation, but in some cases attenuated their intensity. Thus, the increase in cell size, nuclear and nucleolar area, and the presence of varicosities can still be detected.

Quantitative analyses, from 10 sets of experiments and as many as 50 data points per cell characteristic, revealed that cell parameters and their alterations are non-Gaussian in distribution. This large variation in cell size, even in control cultures, requires the use of non-parametric testing, in addition to the Student-t test. We found that both statistical analyses revealed significance in the differences of cell parameters described in Figure 1 after clinorotation ($p < .05$). As a general rule the distribution of almost all cellular attributes has been shifted to the left as a function of clinorotation. Thus, an

increase in myocyte area from a control mean of about 2514 to that of 2876 μm^2 at 5 rpm was noted. In a similar fashion, nuclear area, nucleolar area, and yolk area all show statistically significant increases in rotated cultures. Our initial impression that the number of nucleoli in rotated cultures appears abnormally high (>2) has been borne out by this analysis as well. This is a particularly puzzling finding which indicates nucleolar fissioning, which may be related to the disturbance of equilibrium in the forces acting on the mitotic apparatus. Such an equilibrium has been recently postulated by Bjerknes. While not dealing with such disturbances, it is clearly predictable that cell malformation may result from alterations in such forces. Clinorotation, and by implication microgravity, could easily produce such a deviation from equilibrium. As a final test of the increases in cell area and nuclear area, we examined the percent area of the myocyte occupied by the nucleus. The data show that despite the increase in myocyte area, the increase in nuclear area was slightly disproportionate. Thus, one might expect that an increase in myocyte area would be accompanied by an equivalent increase in nuclear area (to compensate, as it were, for myocyte area increase). The data show that the area of the nucleus has expanded more than the expansion in the myocyte area. This indicates an independent process and further suggests that metabolism per se cannot account for all the changes we have observed.

The highly suggestive hints that cell metabolism, membrane function, cytoskeletal integrity and intracellular organelle structures have been altered led us to hypothesize that a possible common link among these effects might be an altered calcium influx into myocytes during rotation. Such an alteration would have serious ramifications for a multiplicity of cell functions since calcium acts as a universal modulator of many intracellular functions. With this in mind, we explored the possibility of examining the intracellular distribution of calcium in living cells using the FURA2 methods. These pilot experiments were carried out in Dr. Gil Wier's laboratory at the University of Maryland, Baltimore. The salient point of this analysis is that calcium distribution is nonuniform and that this technique can reveal intracellular compartments which may be subject to the clinorotation paradigm. We hope to examine calcium distribution in clinorotated cells with great care during the following year. I believe this analysis could reveal very interesting mechanisms which have already been implicated, from plant studies, to be modulated by microgravity.

Significance of the Accomplishments

The data described above document the effects of microgravitational simulation, using slow clinorotation, on nerve and muscle cells in culture. These myriad of effects is consistent with the interpretation that cell maturation is delayed under these conditions, and that certain cell functions

Figure 1. Effects of clinorotation on the morphology of Xenopus myocytes and neurons grown in culture. Xenopus myocytes and spinal neurons were cocultured for 72 hr in Defined Medium (no serum) and subjected to horizontal clinorotation in the "tumble" position starting at 12 hr after seeding. Rotation rates are indicated at the bottom of the columns. Controls were subjected to the same vibrations and environmental conditions as rotated cultures. Representative micrographs were taken from video frames. The first row shows micrographs from cultures grown in the absence of any media supplementation (0 XEE). The lower rows show micrographs from cultures grown in medium supplemented with 50, 100, or 200 μ l/ml of Xenopus Embryo Extract (XEE), which has been shown by others to promote neurite growth and accelerate cell development. Calibration bar: 50 μ m.

Note that the myocyte area, the nuclear area, and the nucleolar area and number are increased in rotated cultures. Yolk platelet number is also increased in rotated cultures, resulting in an overall increase in the brightness of myocytes. Neurites in rotated cultures develop bizarre varicosities, which are quite different from the normal diamond-shaped enlargements seen in control neurites. The latter are commonly associated with points of attachment or branching.

Effects of Clino-Rotation on Myocyte and Neuron Morphology

XEE=0 μ l/ml

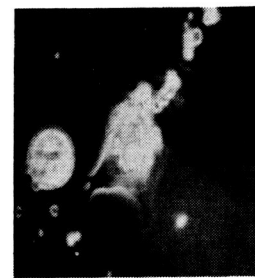


XEE=50 μ l/ml

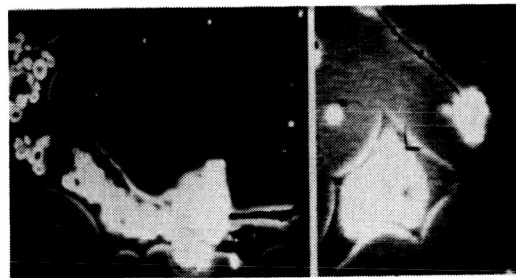


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XEE=100 μ l/ml



XEE=200 μ l/ml



Control

1 rpm

10 rpm

have been adversely altered.

Thus, the late appearance of striations, the retarded consumption of yolk platelets and the production of fewer and thinner neurites indicate subnormal expression of cell characteristics. In addition, these cells do not respond normally to environmental cues such as trophic substances or surface contact enhancers (laminin; data not shown).

How cells perceive alterations in their gravitational field is, of course, not yet known. Nevertheless, based on the effects observed so far, it is possible to propose a working hypothesis. All eukaryotic cells, except plant cells and some unicellular organisms (but see below) contain a subcellular organelle which has been linked to two major functions--cell division and the enucleation of the cytoskeleton of the cell. This organelle, the centriole, is adjacent to the nucleus and appears to be attached to it. The centriole also communicates morphologically with the cell surface via the cytoskeleton. It appears to consist of two orthogonal protein cylinders one of which is parallel to the large surface of cells as they grow in culture (Bornens et al., 1977). On the basis of electron microscopic evidence, Bornens suggested that the centriolar cylinders rotate slowly (about 1 rpm). He further proposed that the centriole acts as the gyroscope of the cell (1979). Bornens suggested that this cellular gyroscope might provide the cell with a kinetic reference point thus controlling cytokinesis, cell shape and size. (These roles would be of particular importance in nerve and muscle cells where cell shape and motility are essential expressions of their function). He also suggested that the centriole would ensure coherence in cell metabolism and that this regulation might be coordinated through the cytoskeleton. Consistent with these ideas, Albrecht-Buehler (1977) has shown that cultured 3T3 cells move in a plane parallel to their centriole. Bornens (1979) therefore concluded that "gravity influences the equilibrium position of the centriole."

More recent studies have shown that the centriole is an essential element in generating and maintaining the cytoskeleton in-vivo (Soltys & Borisy, 1985), as well as in-vitro (Mitchison & Kirschner, 1984). Moreover, time lapse video micrography has proven that the centriole moves and in so doing produces intracellular oscillations in various subcellular organelles including the nucleus (Euteneuer & Schliwa, 1985). Thus, it is logical to conclude that environmental perturbations which might affect the behavior of the centriole will have far reaching consequences in the functional behavior of the cell. Clearly, due to the dynamic nature of the centriole, alterations in the intensity and directionality of gravity can be expected to alter directly cell function.

This hypothesis must, of course, be tested directly. The evidence presented here, and in other studies, while consistent with the hypothesis is still circumstantial. Certain findings,

however, bear specific mention as they appear to be crucial. First, the alterations in nuclear and nucleolar size, reported here, can be predicted to occur if the integrity of the centriole has been affected by clinorotation. Second, the relative disorganization of membrane-bound receptors is similarly expected if, secondary to alterations in the centriole, the structure of the cytoskeleton is also compromised. This is so because many membrane-bound proteins, including the acetylcholine receptor, have been shown to be stabilized somehow by the cytoskeleton (Bloch, 1983). Third, results reported in this study from clinorotated cells should be consistent with data reported from direct interference with the integrity of cytoskeletal elements. Indeed, it is of interest to note that Letourneau & Ressler (1984) showed that exposure to taxol, which promotes microtubular polymerization, produces microtubular tangling and looping which appear as large varicosities at neuritic terminals. The neuritic varicosities described here may have a similar origin. Similarly, Domnina et al. (1985) reported that epithelial cells exposed to cytochalasin and colcemid, agents which destroy microtubules, produce thin cytoplasmic extensions which appear to contain prominent varicosities. Finally, the absence of the centriole in plant cells raises the enigma of their sensitivity to alterations in gravitational forces. It is of great interest to note here that Clayton et al. (1985) found by immunofluorescence that spindle poles of meristematic higher cells are recognized by a human serum containing antibodies to pericentriolar microtubular organizing centers. Moreover, during mitosis this fluorescent probe is redistributed in the plant cells in such a fashion that these authors conclude that "perinuclear region, or the nuclear surface, may function as a nucleation center" for microtubular structures found in higher plants. The similarity to eukaryotic animal cells is self evident.

In summary, I have shown that clinorotated cells display morphologic alterations which are consistent with the hypothesis that altered gravitational forces may act via the cell's centriole. Such action may be mediated via surface events such as calcium influx which may then modulate cytoskeletal restructuring. The observations reported here bear strong, albeit circumstantial, resemblance to those reported to occur consequent to interfering with centriolar and cytoskeletal integrity. Such a resemblance may be ascertained by direct testing of the proposed hypothesis.

THE EFFECTS OF HYPERGRAVIC FIELDS ON NEURAL SIGNALLING IN THERMOREGULATORY AND VESTIBULAR SYSTEMS IN THE RAT

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Description of Research

Experiments were conducted on thermoregulatory and vestibular function in rats. The studies centered on the neural processing of sensory information. They were designed to continue investigation of alterations in the function of the central nervous system in animals exposed to Earth gravity and to hypergravitational fields. For example, one study centered on the response of brainstem nuclei in the vestibular system. Using methods previously developed (Soc. for Neurosci. Abs. 10: 112, 1984), responses were recorded for impulse accelerations. Data indicate that in rats one can evoke far-field responses due to activity over the vestibular system and that this activity can be clearly distinguished from auditory evoked responses.

In addition to these studies, many of our experiments were related to a striking experimental finding from Spacelab-3, namely that there is an elevation in serotonin binding in the hippocampus of rats exposed to microgravity for 7 days. This observation led us to focus on developing techniques to study a specific region of the central nervous system, the hippocampus, where the changes in receptor number have been reported. The hippocampus is an area of the central nervous system that appears to be involved in memory and learning. The goal of the experiments was to lay the basis for determining functional changes in the hippocampus in rats and hamsters exposed to hypergravic fields.

Slice techniques were developed to study the effects of bath temperature and pH on evoked electrical activity. The hippocampus was sliced and placed in chilled artificial cerebrospinal fluid (ACSF). Slices 400-450 μ thick were cut perpendicular to the long axis of the hippocampus and placed in a holding chamber. Approximately 1-2 hours after tissue dissection, a slice was transferred from the holding chamber to the submerged, constant-perfusion slice chamber. The slice was submerged in ACSF between two nets, the top net serving to hold the slice in place. The tissue was constantly perfused with oxygen-saturated ACSF at a rate of 1.5 to 2 ml/min via a gravity-fed reservoir system. The ACSF was removed by suction from a second chamber located behind and connected to the recording chamber. Temperature was monitored via a calibrated thermistor placed in the recording chamber next to the slice. Action potentials from a population of hippocampal pyramidal neurons were evoked by stimulating an afferent fiber tract, the

Schaffer collaterals. The temperature and the pH of the ACSF bathing the slice were varied by controlling the temperature of a water chamber jacketing the recording chamber.

Accomplishments

The major findings of this study are:

(1) The frequency spectrum of bone-conducted vibrations coupled to the skull of rats during impulse angular acceleration stimulation was estimated to have greatest power at 2-3 kHz. The intensity of these vibrations was approximately 5 dB lower than the vibrations evoked by bone-conducted auditory clicks, which had their greatest power between 9 and 11 kHz.

(2) The effects of pH and temperature on hippocampal population spikes were determined. Thresholds for evoked activity were significantly different in noncold-acclimated, cold-acclimated and hibernating hamsters, reflecting acclimation of hippocampal neurons to cold.

(3) Plots of population spike amplitude (action potentials from a group of pyramidal cells) versus temperature have bell-shaped curves. The population spikes increased in amplitude as temperature was lowered from 35°C, reached a peak amplitude between 25 and 20°C, and then decreased until a response could not be evoked when temperature was further lowered.

(4) A model was developed to predict the effect of temperature on the electrical activity of a hippocampal pyramidal cell. Four populations of membrane channels in the pyramidal cell were simulated.

Significance of the Accomplishments

Finding #1: Data confirmed a previous report that noninvasive procedures can be used to record brainstem vestibular evoked responses in rats. Particular attention was devoted to ruling out the possibility that what had been called a vestibular response was in reality an auditory response. Thus stimulus characteristics were precisely determined. Using this type of stimulation, the amplitudes of the first two major components of the response evoked by angular acceleration were greater than the first two major components of the response evoked by bone-conducted clicks. Thus at present two independent laboratories have reported brainstem vestibular responses.

Finding #2 and #3: These experiments provided information on the effects of temperature and pH on hippocampal electrical activity. The response of the neural network was found to be dependent on acclimation of the animal to ambient temperature. Thus the threshold for hibernating hamsters was lower than for noncold-acclimated hamsters. Moreover, the amplitude of the population spike versus temperature showed that this amplitude reached a peak at 20 to 25°C. These studies more fully describe temperature-dependent function in the hippocampus. Experiments show that for pH and temperatures within the range found in awake and hibernating hamsters, the changes in waveforms as pH was

varied were much smaller than the changes observed as temperature was varied. These experiments provide the basis for our planned experiments on serotonin.

Finding #4. With the development of brain slice techniques for voltage-clamping single cells, several types of ion channels in membranes of mammalian nerve cells have been identified and characterized in sufficient detail to allow a simulation of channel currents. Modeling of these currents provides information regarding the contribution of each channel type to the response of the nerve cell to incoming signals. We have simulated the effect of temperature on channels in hippocampal cells as a step in studying the mechanisms by which temperature alters the processing of neural signals.

Equations for current through the ion channels are similar to those first developed by Hodgkin and Huxley for sodium and potassium channels in the squid axon and more recently extended by Traub to include not only these channel types but, in addition, calcium and calcium-mediated potassium channels in hippocampal cells. Voltage and/or concentration-dependent rate functions were used to describe the kinetic behavior of each population of channels. A temperature-dependent term was included for each rate function to simulate the effect of changing temperature on neural activity. Model simulations correspond to experimental data over a range of temperature from 40°C to 35°C.

Publications

Thomas, M.P., Eckerman, P.J., and Horowitz, J.M. Temperature and pH Effects on Evoked Responses from Hamster Hippocampal Slices (Abstract). Society for Neuroscience, 1986.

Thomas, M.P., Martin, S.M., and Horowitz, J.M. Temperature Effects on Evoked Potentials of Hippocampal Slices from Noncold-acclimated, Cold-acclimated and Hibernating Hamsters. Journal of Thermal Biology 11: 213-218, 1986.

MICROGRAVITY-INDUCED EFFECTS ON PITUITARY GROWTH HORMONE CELL FUNCTION

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Description of Research

The long-range goal of the research is to determine the extent to which mammalian pituitary growth hormone (GH) cell function may be affected in spaceflight.

Specific objectives include establishing, characterizing, and validating a closed vial pituitary cell culture system for a future flight experiment.

Mammalian GH regulates a number of key biological processes. For example, GH (a) increases skeletal growth, (b) increases protein synthesis, (c) decreases carbohydrate uptake into cells, (d) increases breakdown of fat, and (e) is a mitogen. Since GH controls these metabolic activities in muscle and bone, tissues known to be affected by spaceflight, it is important to determine if the mechanisms governing synthesis and secretion of GH are modified in microgravity. The problem is complicated by the fact that a number of GH variant forms are known to exist in the pituitary gland. The biological activities of these variants also differ. Evidence from our laboratory suggests that intracellular processing of GH variants may be unique in different subpopulations of GH cells. Finally, it is likely that the nature of the GH molecules being released from different GH cells is under physiological control (e.g., brain peptides/amines). The overall thrust of our research is therefore to: (a) examine mechanisms of intracellular processing and secretion of GH variants, (b) improve our ability to detect biologically active GH forms secreted from GH cells in culture, and (c) identify molecules coming from the brain which may regulate these activities.

Experiments Conducted:

- (1) Tests of the closed vial culture system to establish cell viability for future flight experiment
- (2) Closed vial vibration test
- (3) Test comparing pituitary GH cell function from unloaded rats on Earth vs. those previously established on pituitary cells of rats flown on the SL-3 mission (April, 1985).

Accomplishments

(1) Results to date establish that cells in the closed vial system are alive after a 9-day test period. Specifically, they show that:

- (a) GH release from cells attached to the vial surface is linearly related to cell dose (up to 8×10^5 cells seeded)
- (b) the amount of GH synthesized is typically 15-20 μg for 2×10^5 cells seeded
- (c) GH synthesis in the closed system compares favorably with that measured in cells maintained on conventional plastic substrates in open systems
- (d) intact, viable cells can be recovered from the vial after the 9-day culture and
- (e) GH release from 500 μm sections of living pituitary tissue can also be maintained in viable state for 9 days. This enables us to investigate the critical variable of cell-cell interactions in relation to GH secretion.

(2) A successful vibration test of the cell culture flight hardware was conducted at NASA Ames Research Center. Vials were exposed to vibrations equivalent to a 1981 Shuttle launch profile. Results showed that there was no (a) vial breakage, (b) leakage of media, (c) cell detachment from the vial surface, or (d) effect on GH release.

(3) The SL-3 mission flew rats for 7 days. Analysis of the pituitary GH cells from those rats showed that, relative to ground based controls:

- (a) they contained more intracellular hormone
- (b) their absolute numbers were similar
- (c) they released less GH into the culture medium on return to Earth
- (d) they released less GH upon reimplantation into hypophysectomized rats
- (e) they were apparently unable to release a particularly potent, high molecular weight form of the hormone in vitro.

The SL-3 experiment was mimicked on Earth in February 1987 (under the direction of Dr. E. Holton). Rats were "unloaded" in the RAHF for 7 days and pituitary cells prepared for experimental protocols identical to those on SL-3. Relative to cells from unloaded rats kept in the RAHF for 7 days we found: (a) more intracellular GH in "flight" cells, (b) no change in GH cell numbers, (c) suppression in release of GH in vitro, and (d) suppression in release of GH in vivo (reimplantation into hypophysectomized hosts). These results therefore reproduced those of the actual flight experiment and point to the utility of the "unloaded" rat to simulate microgravity.

Significance of the Accomplishments

The results of our analyses of pituitary GH cell function in rats flown in microgravity indicated that several important changes occurred relative to cells prepared from animals kept on the ground. In sum, we believe that the changes reflect the existence of a micro-g "secretory lesion" in at least some of the GH cells. It is emphasized that the "shutdown" is not a total one; that is, some GH was released from cells of flight animals (SL-3). Little is known regarding the mechanism of this effect. It may reflect adaptation to a new environment. The fact that the flight cells were not as effective in reinitiating animal growth on Earth is consistent with the hypothesis that the flight cells may not be as responsive to stimulatory agents from the brain. In adapting to the new environment, is it possible that microgravity signals are "sensed" by tissues (such as muscle, bone, brain) which, in turn, "inform" the pituitary of the need for an adaptive change? Or, on the other hand, is it possible that microgravity has direct effects, i.e., is the lack of gravity sensed directly on the pituitary GH cell itself? The results of our preliminary cell culture experiment done on the STS-8 mission suggested that this latter possibility might indeed be correct. In this respect, our positive findings relating to the development of a cell culture system designed to accomodate large numbers of samples (to ensure statistical validity) are important.

Finally, the results of the recent SL-3 simulation experiment are particularly encouraging in that the direction and order of magnitude of the changes in GH cell function between actual and simulated flight are similar.

Publications

Grindeland, R., Hymer, W.C., Farrington, M., Fast, T., Hayes, C., Motter, K., Patil, L., and Vasques, M. Changes in Pituitary Growth Hormone Cells Prepared From Rats Flown on Spacelab 3. American Journal of Physiology 252: R209-R215, 1987.

MECHANISMS OF VESTIBULAR ION TRANSPORT

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Description of Research

The two classes of vestibular sensory organs, the maculae and ampullae (Figure 1), differ by virtue of the accessory structures which mediate their respective mechanical sensitivities to linear or angular acceleration. In contrast, the mechanoreceptive hair cells associated with each type of receptor organ show many similarities of organization and function within the sensory epithelia. The hairs of the receptor cells, in each type of structure, extend toward the lumen of a fluid compartment filled with endolymph. This specialized extracellular fluid is distinguished by its unique low-sodium/high-potassium ionic composition. The ionic profile of endolymph holds special significance for hair cell function. Vestibular transduction is thought to commence when mechanical displacement of the sensory hairs results in the opening of "transduction channels" at the apical cell surface, with augmented flow of ionic current from endolymph into the hair cell. The transduction current is carried, in mammalian hair cells, almost entirely by endolymphatic potassium ion.

The endolymphatic compartments of the vestibular and auditory systems comprise an array of interconnected chambers, which together approximate a closed system bounded by heterogeneous epithelial walls (cf. Figure 1). Electrochemical considerations indicate that the steep gradient of potassium concentration between the endolymph and other extracellular fluids must result from active transepithelial transport of potassium ion. The long-term objectives of this research are to identify the sites of active ion transport in vestibular tissues, and to study possible mechanisms regulating the rate of transport.

The mechanisms responsible for maintenance of endolymphatic ion content may also participate in the regulation of endolymphatic volume. Available evidence indicates that the osmotic pressure of the endolymph is determined almost entirely by its inorganic ionic constituent, and that membranes of the endolymphatic compartment are freely permeable to water. The endolymphatic compartments may consequently gain or lose water in the presence of an osmotic gradient between the endolymph and surrounding extracellular fluids.

Initial exposure to microgravity is accompanied by a documented redistribution of extracellular fluid from the lower body to the thoracic region, and it has been suggested that subsequent

MAMMALIAN INNER EAR

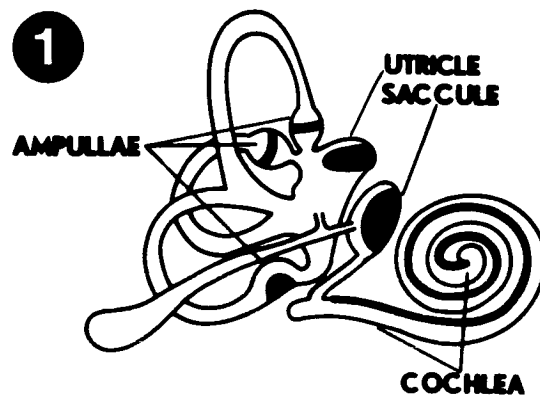


Figure 1. This diagram provides a schematic representation of the various inner ear endolymphatic compartments. The utricle, saccule, and ampullae are components of the vestibular apparatus, while the cochlea mediates the sense of hearing. The locations of the sensory epithelia within the endolymphatic compartments is indicated by black shading.

adaptation to weightlessness may involve transient compensatory alterations in whole-body fluid and electrolyte balance. It is therefore important to determine whether the endolymphatic compartments are involved in these processes, and whether the vestibular apparatus may incorporate independent homeostatic mechanisms for regulation of endolymphatic ion transport and fluid volume.

The only putative mechanism for transepithelial potassium transport yet demonstrated in vestibular tissues is the enzyme Na^+, K^+ -ATPase. Previously we utilized autoradiographic and cytochemical methods to determine the enzyme's distribution in the utricle and ampullae of the albino guinea pig. High levels of Na^+, K^+ -ATPase were detected in specialized epithelial cells termed dark cells, which form circumscribed epithelial patches in the ampullae and the wall of the utricle.

In pigmented mammals, melanocytes (pigment cells) are distributed subjacent to the epithelial walls of the utricle and ampullae. Moreover, the distribution of pigment cells closely parallels the distribution of the dark cells. A similar anatomical relationship between presumptive ion-transporting cells and melanocytes exists also in the cochlea. Differences in the auditory responses of pigmented vs. albino mammals have been reported, and some investigators believe that the melanocytes may play a part in regulation of inner ear fluid balance. Since our future studies will utilize the gerbil as an experimental subject, we have, in the first months of the project, compared the distribution of vestibular Na^+, K^+ -ATPase in this pigmented species to that which we previously observed in the utricle and ampullae of the albino guinea pig. In addition, we have examined the epithelial wall of the saccule for possible elevated enzymatic activity indicative of transepithelial ion transport.

The cytochemical technique used in these studies detects inorganic phosphate, released from an artificial substrate (nitrophenyl phosphate) by catalytic activity of the enzyme. In the presence of strontium ion, phosphate is precipitated near regions of high Na^+, K^+ -ATPase activity, then converted to a product which may finally be visualized in the electron microscope.

Accomplishments

(1) These studies demonstrated that dark-cell epithelium in the utricle (Figure 2) and ampullae of the gerbil displays intense Na^+, K^+ -ATPase activity. Moreover, the distribution of enzymatic activity is asymmetrical. Nearly all enzymatic reaction product is restricted to the complex basolateral membrane extensions of the dark cells, with little or no activity at the luminal surface facing the endolymph (Figure 2).

(2) It was found that melanocytes are present immediately subjacent to the reactive dark-cell basolateral membrane extensions. The melanocytes send out "tendrils," or cellular

NA⁺, K⁺-ATPase ACTIVITY IN MAMMALIAN INNER EAR



Figure 2,3, and 4. These electron micrographs illustrate tissues of the gerbil inner ear following cytochemical incubation to demonstrate Na⁺, K⁺-ATPase activity.

Figure 2. This micrograph illustrates a segment of the "dark cell" epithelium in the wall of the utricle. The nucleus of a dark cell (D) is at upper right. In the basolateral extensions of the dark cells, copious deposits of reaction product (arrowheads) reflect intense Na⁺, K⁺-ATPase activity; the endolymphatic surface shows little or no reaction. Cellular extensions of melanocytes, filled with prominent pigment granules, are closely apposed to the basal surface of the dark cell epithelium, and to a capillary (C). Neither melanocytes nor capillary endothelial cells show appreciable enzymatic activity. EL: endolymph. Calibration: 5 μ m.

NA⁺, K⁺-ATPase ACTIVITY IN MAMMALIAN INNER EAR

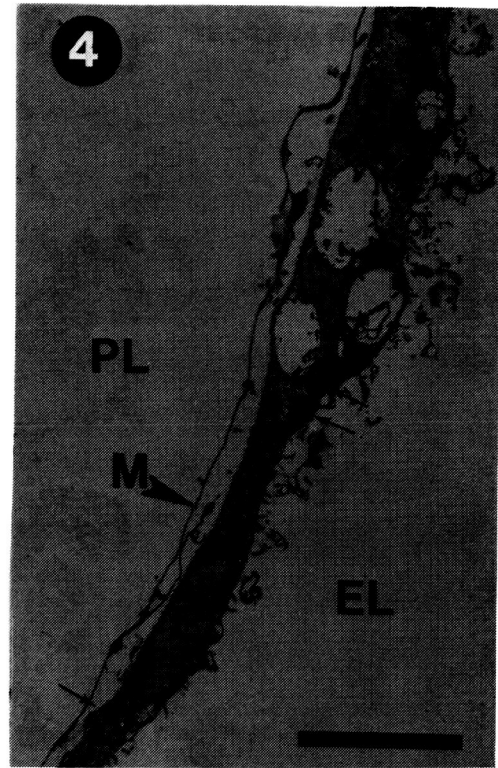
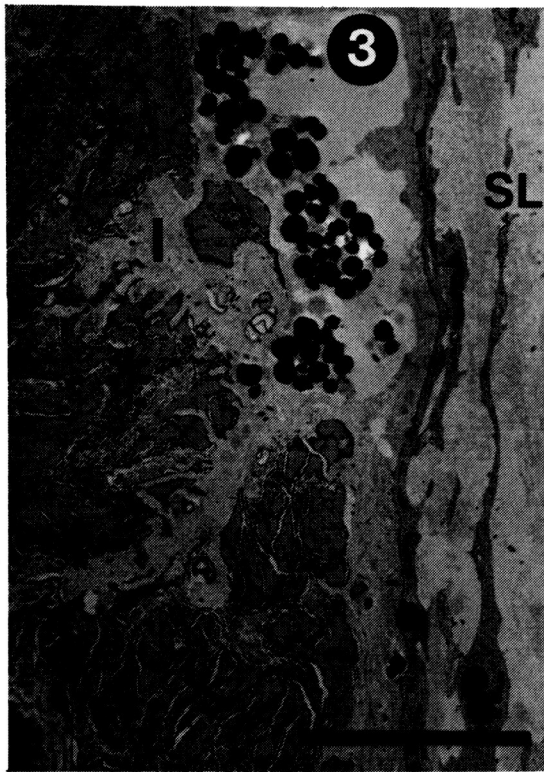


Figure 3. This micrograph shows the basal portion of the stria vascularis from the gerbil cochlea. Clusters of marginal cell extensions (*), with prominent deposits of reaction product, are separated by the non-reactive processes of an intermediate cell (I). Clusters of pigment granules (uppermost center of the field) seem to be associated predominantly with the intermediate cell. The endolymphatic compartment lies outside the field of view, at left. SL: Spiral ligament (connective tissue). Calibration: 5 μ m.

Figure 4. This micrograph shows a portion of the free wall of the saccule. Although a few isolated pigment granules (arrows) are present in the epithelial cell layer (E), no enzymatic reaction product can be observed, either in the epithelial cell layer or in the mesothelial (M) layer. EL: endolymph; PL: perilymph. Calibration: 5 μ m.

extensions, containing clusters of pigment granules. These are found closely apposed not only to the basolateral surfaces of dark cells, but also to the numerous capillaries distributed beneath the dark cell epithelium (Figure 2).

(3) There is no readily apparent difference in regional distribution of inner ear Na^+ , K^+ -ATPase activity within presumptive ion transporting epithelia of the pigmented gerbil or the albino guinea pig. In addition to the dark cells of the utricle (Figure 2) and ampullae, the analogous marginal cells of the cochlear lateral wall show intense activity along their basolateral membrane surfaces (Figure 3). As in vestibular dark-cell epithelia, pigment cells (termed "intermediate" cells) are closely related to the cochlear marginal cells (Figure 3), and to adjacent blood vessels.

(4) No enzymatic reaction product has been observed in the free epithelial wall of the saccule (Figure 4). Aside from the striking difference in Na^+ , K^+ -ATPase activity in saccular wall vs. dark-cell epithelium of utricle and ampullae, several other differences may be noted: (a) saccular epithelial cells lack the complex basolateral membrane infoldings characteristic of the dark cells; (b) the free saccular wall is avascular; and (c) no melanocytes are present beneath the saccular epithelium, although a few small pigment granules are present in the epithelial cells themselves (Figure 4).

Significance of the Accomplishments

Finding #1. Na^+ , K^+ -ATPase is ubiquitous in mammalian cells; the enzyme participates in the maintenance of the high K^+ /low Na^+ intracellular ionic environment by active inward transport of K^+ , and outward transport of Na^+ , across the plasma membrane. The presence of elevated activity in particular cell types, however, constitutes presumptive evidence that the enzyme serves a specialized cellular function. Moreover, the asymmetrical, or "polarized," distribution of reaction product demonstrated in vestibular dark cells is a hallmark of epithelial cells engaged in transepithelial transport of K^+ and/or Na^+ . The distribution of enzymatic activity along the basolateral membrane of the dark cell is compatible with a mechanism involving active uptake of K^+ from the "blood" side of the epithelium, with subsequent transport into the endolymphatic compartment.

Findings #2 and #3. These observations show that inner ear epithelial cells implicated in potassium transport on the basis of their high Na^+ , K^+ -ATPase activity always occur in conjunction with subepithelial capillaries, and (in pigmented mammals) melanocytes. The rich vascularization of vestibular dark cells and cochlear marginal cells probably serves for the most part to furnish oxygen and nutrients needed for synthesis of the enzymatic substrate ATP.

The possible significance of the melanocytes for epithelial transport processes remains enigmatic. There was no apparent difference in the distribution of Na^+ , K^+ -ATPase in vestibular

dark cells or cochlear marginal cells from albino or pigmented animals. Our results therefore provide no evidence for a direct functional relationship between ion-transporting epithelial cells and melanocytes (although they do not exclude such a relationship). It has previously been suggested that the melanocytes may exert a vasomotor influence on the subepithelial capillaries. This mechanism might lead to an indirect modulation of endolymphatic ion transport by regulation of ATP synthesis in the epithelial cells (see above).

Finding #4. The lack of elevated Na^+ , K^+ -ATPase in the epithelial wall of the saccule (Figure 4) strongly suggests that the electrochemical profile of saccular endolymph is maintained by transport sites in one of the other endolymphatic chambers. There is anatomical evidence to suggest that the narrow passage from utricle to saccule (Figure 1) prevents free exchange of endolymph between these two compartments. At the same time, electrophysiological results indicate that the electrochemical profile of saccular endolymph depends on the integrity of the cochlea. Our own observations demonstrate intense Na^+ , K^+ -ATPase in the marginal cells of the cochlear lateral wall (Figure 3). Taken together, these considerations indicate that cochlear ion transport mechanisms play a critical role in the function of the saccule.

Publications

Keir, T.P., Ross, M.D., and Ernst, S.A. Cellular Localization of Na^+ , K^+ -ATPase in the Mammalian Vestibular System (Abstract). Association for Research in Otolaryngology, Abstracts (Ninth Midwinter Research Meeting), 115, 1986.

HIGH RESOLUTION ANALYSIS OF GRAVITY ORIENTATION OF AMPHIBIAN EGG CYTOPLASM

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Description of Research

Following activation by a fertilizing sperm, the amphibian egg displays a dramatic gravity orientation, which is easily visualized because of pigmentation differences between regions of the egg surface. Internally, relatively large yolk platelets provide a key marker for the cytoplasmic rearrangements which accompany gravity orientation. Studies under microgravity simulation (clinostat), egg inversion, or spaceflight can be employed to understand the basic cell biology of the organization of the egg cytoplasm. The goals of recent experimentation have been directed towards understanding the forces and mechanisms which are responsible for maintaining the structural integrity of the various compartments (see below). Also, the mechanisms which are responsible for rearranging the compartments are being studied.

Accomplishments

Previous results have revealed that the egg cytoplasm is organized into a series of domains or "compartments," which differ from one another with regard to yolk content. The rearrangements of those compartments which follow fertilization are believed to generate the bilateral symmetry of the egg and body plan of the embryo. In order to understand the physical forces which provide structural integrity to individual compartments, and which facilitate compartment reorganization, the yolk platelet packing characteristics of the various compartments have been analyzed. Eggs have first been characterized according to their intrinsic cytoplasmic mobility (high mobility cytoplasm; intermediate mobility cytoplasm; low mobility cytoplasm), and then the yolk platelet packing density measured. Two alternative hypotheses have been tested: (a) packing differences account for compartment features; and (b) cytoskeletal differences, rather than packing differences, account for compartment features. The data is, so far, consistent with the latter hypothesis. Initial results indicate that yolk platelet packing differences are relatively similar among different (HMC, IMC, LMC) egg types.

Several details can be summarized as follows:

- (1) Large numbers of spawnings were tested for mobility of the egg cytoplasm by determining the degree of large yolk compartment movement in eggs that were inverted with respect to

the gravity vector. Unfertilized and fertile eggs from spawnings that showed the higher, lower, as well as the intermediate cytoplasmic mobilities were fixed, embedded in plastic, and sectioned at 0.5 and 1.0 μ m. A wide range in cytoplasmic mobilities was documented (16.9 units to 72.7 units).

(2) The proportion of the area of the yolk compartments taken up by yolk platelets (packing density) was determined by quantitative morphometrics. The packing density of the yolk compartments was as follows: large yolk mass > intermediate yolk mass > small yolk mass.

(3) No direct relationship between packing density and cytoplasmic mobilities was discovered. For example, the mean packing density of the large yolk mass in a batch of high mobility cytoplasm (18.6 units) eggs was 0.6662, while the mean packing density of a batch of low mobility cytoplasm (68.8 units) eggs was 0.6581.

Significance of the Accomplishments

The main goal of this year's accomplishments is to understand the features of cytoplasmic organization which are relevant to the egg's response to gravity orientation following fertilization. That is, data are being collected which will help answer two questions:

- (1) How does the amphibian egg manage to maintain the stratification of its major cytoplasmic components (e.g., yolk platelets), according to buoyant density, prior to fertilization?
- (2) What mechanisms rearrange the buoyant density compartments during egg activation (fertilization)?

STRUCTURAL DEVELOPMENT AND GRAVITY

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Description of Research

The ultimate goal of this research is to understand the role of gravity in skeletal growth and development. To achieve this goal, we must first learn what turns bone cells on and off, if/how these cells communicate with each other and with their environment, how alterations in structural matrix might change mineral crystal size and/or composition which, in turn, might alter bone stiffness, and whether changes are induced by systemic or by local mechanisms. To accomplish these goals, both ground-based and flight experiments are essential.

Gravity is a major factor determining the amount of structural support required by Earth-bound organisms. The hypothesis of this research effort is that skeletal support structures will change during spaceflight and that the degree of change will be dependent upon the growth rate of the bone and the length of exposure to flight; changes in both quality and quantity of bone will occur. Most ground-based research is done in rats exposed to simulated spaceflight; three flight experiments have been approved and will allow gathering of more information to support or negate the hypothesis.

Accomplishments

The major findings during 1986 were:

- (1) Tenotomy studies in unloaded rats indicate a significant decrease in periosteal bone formation (PBF) at the tibiofibular junction (TFJ) in the tenotomized-unloaded group compared to the sham-operated unloaded group, suggesting that muscle tension partially protects bone growth during unloading.
- (2) Epiphyseal plate width in the proximal tibia of growing rats decreased with age; a 42% reduction in width was found between 6 and 11 weeks of age, followed by a 36% decrease from 11 to 20 weeks, while from weeks 20 to 69 the growth plate width remained constant at 37% of the initial width.
- (3) Initial studies for an SL-3 simulation suggest that suspended rats need slightly more floor space than do animals in a RAHF-simulated cage to gain weight similarly.
- (4) SL-3 studies were completed and showed a significant suppression of PBF at the TFJ in the older flight animals, while metaphyseal parameters, although trending toward suppression, were not significantly different.

Significance of the Accomplishments

Finding #1 suggests that muscle pull partially protects against

decreased bone growth during mechanical unloading of long bones. However, decreased muscle tension and unloading may affect similar growth mechanisms since the responses of bone to tenotomy and to unloading were not purely additive when applied simultaneously, i.e., tenotomized rats showed a 34% suppression of bone formation while sham-operated unloaded rats decreased bone growth by 28% and tenotomized unloaded rats showed a 42% suppression of formation rather than 62% suppression; this would be anticipated if muscle tension and loading were operating through different mechanisms and were additive.

Finding #2 suggests that longitudinal growth of bone in rats decreases rapidly with age; by 20 weeks of age, the width of the growth plate was only 1/3 that at 6 weeks of age. This finding agrees with others in the literature that document that rats, like humans, grow in length only during the growth period of the animal.

Finding #3 showed that unloaded rats cannot manipulate throughout the entire volume of their cage and thus require more floor space to grow normally than do normal, weightbearing rats.

Finding #4 documented that significant suppression of bone growth occurred within 7 days of spaceflight in the older group of rats (no bones with bone markers were available from the younger animals).

Publications

Arnaud, S.B., Schneider, V.S., and Morey-Holton, E. Musculoskeletal Changes Induced by Bed Rest. In: Inactivity: The Physiology of Bed Rest (ed. by H. Sandler and J. Vernikos-Danellis). New York: Academic Press, pp. 49-76, 1986.

Fiedler, P.J., Morey, E.R., and Roberts, W.E. Simulated Weightlessness Disrupts Osteoblast Histogenesis in Periodontal Ligament and Tibial Metaphysis. Aviation, Space and Environmental Medicine 57: 1125-1130, 1986.

Halloran, B.P., Bikle, D.D., Levens, M.J., Castro, M.E., Globus, R.K., and Holton, E. Chronic 1,25-dihydroxyvitamin D Administration in the Rat Reduces the Serum Concentration of 25-hydroxyvitamin D by Increasing Metabolic Clearance Rate. Journal of Clinical Investigation 78: 622-628, 1986.

Roberts, W.E., Weinreb, M.W., Gonsalves, M.R., and Morey, E.R. Nuclear Volume Assay of Osteogenic Potential in Ulna Primary Spongiosa of Rats Flown in Space for 18.5 Days. Anatomical Record 214: 109A, 1986.

Wronski, T.J., Halloran, B.P., Bikle, D.D., Globus, R.K., and Morey-Holton, E.R. Chronic Administration of 1,25(OH)₂D₃: Increased Bone but Impaired Mineralization. Endocrinology 119: 2580-2585, 1986.

Wronski, T.J. and Morey-Holton, E.R. Skeletal Response to Simulated Weightlessness: A Comparison of Suspension Techniques, Aviation, Space and Environmental Medicine 58: 63-68, 1987.

Wronski, T.J., Morey-Holton, E.R., Doty, S.B., Maese, A.C., and Walsh, C.C. Histomorphometric Analysis of Rat Skeleton Following Spaceflight. American Journal of Physiology 252: R252-R255, 1987.

HYPERGRAVITATIONAL EFFECTS ON MAMMALIAN FETAL AND NEONATAL GROWTH AND DEVELOPMENT

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Description of Research

The long-range goal of this project is to develop a better and more comprehensive understanding of the role and directive influence of terrestrial gravity on mammalian growth and development, particularly during the gestational and birth to weaning periods, through studies which encompass the whole range of gravitational environments ranging from "zero-g" and fractional-g to hyper-g.

Current research is focused on long-duration (weeks/months) hyper-g effects using chronic centrifugation as a means of studying animal development in different and graded hyper-g intensities. Studies are conducted under conditions in which only the g-intensity is varied and the directional vector is maintained constant.

The objectives of the studies are to: (a) delineate the most significant structural and functional changes and determine when they occur; (b) establish quantitative relationships between the induced developmental changes and g-intensity, including the determination of g-thresholds; (c) evaluate the scaling effects of body size differences on gravitational effects from interspecies comparisons; and (d) develop hypotheses which can be tested in future space experiments dealing with animals in "zero-g" and in fractional-g (in-flight centrifuge).

During the past year, the research has focused on the guinea pig, with studies extending the range of g-intensities previously employed to investigate the effects of hyper-g on reproduction, prenatal and postnatal growth, and morphological development. The initial study at 1.27 g and 1.71 g was expanded to 1.48 g and 2.03 g. Gross morphological measurements of the skeletal and muscular systems in 70-72 day-old guinea pigs at 1.27 g and 1.71 g were completed and comparisons made with results obtained on rats. Studies were initiated to investigate the effects of hyper-g on guinea pig fetuses at definite time periods of gestational development after successfully demonstrating that timed-matings could be performed under hyper-g conditions.

Accomplishments

(1) Chronic centrifugation experiments have shown that hyper-g adapted guinea pigs are able to successfully mate,

undergo gestation, and deliver viable litters at 2.03 g, the highest g-intensity employed so far.

(2) Survival rates of newborn guinea pigs in hyper-g were found to be significantly higher than for rats at comparable g-intensities. At 2.03 g, for example, approximately 82% of the newborn guinea pigs survived compared to 37% for rats.

(3) Bone/body mass ratios of the femur and tibia-fibula of guinea pigs, conceived and reared at 1.27 g and 1.71 g for 70-72 days, were found to be significantly higher than 1 g controls: females' ratios were 7-10% and males 14-18% higher.

(4) No significant mass changes in soleus muscle were found between the 1 g control and 1.27 g and 1.71 g guinea pigs. No consistent or general effect attributable to hyper-g loading was found in any of the remaining hind limb muscles, which is similar to previous findings in hyper-g-rats.

(5) Preliminary timed-mating studies of guinea pigs have shown no significant changes in organ/body mass ratios of 58 day-old fetuses at 2.03 g. Timed-matings were conducted by remating mothers within 12-18 hr after delivery of their first litter (post-partum estrus).

Significance of the Accomplishments

Results of reproduction studies in hyper-g show that newborn guinea pigs survive better than smaller-sized rats. These results point out the fact that while organism size is one of the critical factors affecting development, it is not the determining factor in the survival of newborn animals in hyper-g. The higher survival rate of newborn guinea pigs is undoubtedly due to their reaching a more advanced stage of development during gestation, compared to the rat. The gestational period of the guinea pig (approximately 65 days) is about three times as long as the rat. At birth, the guinea pig has a body size of approximately 100 grams, or approximately 20 times the newborn rat and is fully covered with hair. The latter factor greatly enhances the ability of the newborn guinea pig to reduce its rate of heat loss and maintain its body temperature as compared to the hairless newborn rat.

Results from prenatal studies, in which significant decreases in fetal growth rates have been effected by hyper-g exposures, lend strong support for use of the guinea pig as a model for more extensive and detailed studies on the role of abnormal gravitational fields on animal growth and development. The mass increase from the fertilized egg to the end of gestation (100 grams) provides a useful range for investigating the scaling effects of body size on gravitational effects. The range is particularly appropriate for investigating how large an animal must be before hyper-g can significantly and permanently affect its growth and development. Another appealing aspect is that growth and development proceeds under relatively stable and constant environmental conditions maintained and regulated by the mother.

As yet there is no clear cut explanation for the absence of any significant increase in the size (mass) of any of the limb muscles of hyper-g adapted rats and guinea pigs. Significant increases in bone/body mass ratios have been found in both rats and guinea pigs in hyper-g. These contrasting results indicate that skeletal development is either stimulated or proceeds at a normal rate in hyper-g. However, there is a significant decrease in the growth rate of muscle tissues. The decrease in limb muscle mass parallels the decrease observed in the overall body mass and other soft tissues so that the muscle/body mass ratios are unchanged in hyper-g animals, when compared to controls. Lower levels of physical activity of hyper-g animals as compared to 1 g animals may possibly account for the lack of any significant muscle mass increases.

Publications

Mednieks, M.I., Fine, A.S., Oyama, J., and Philpott, D.E. Cardiac Muscle Ultrastructure and Cyclic AMP Reactions to Altered Gravity Conditions. American Journal of Physiology 252: R227-R232, 1987.

BONE CELL KINETICS OF SIMULATED WEIGHTLESSNESS

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Description of Research

The osteogenic (bone forming) components of bone modeling (change in size and/or shape) and probably remodeling (turnover of preexisting bone) are inhibited by decreased skeletal loading and/or microgravity. The long range goals and objectives of this research are: (a) to define the cellular mechanism of osteoblast (bone forming cell) production and (b) to determine how this process is suppressed in microgravity. Spaceflight is the only experimental means known for probing the gravity dependence of osteoblast production.

The present cell kinetics research utilizes DNA labeling (^3H -thymidine), mitotic activity, and nuclear size as indices of the proliferation and differentiation aspects of osteoblast histogenesis (production of bone forming cells). The central thrust of these studies is to determine the relative influence of gravity, mechanical loading, and physiological stress on osteoblast histogenesis in weight bearing bones (tibia, ulna, etc.), a non-weight bearing bone (maxillary molar periodontal ligament), and an antigravity postured bone (mandibular condyle).

Previous research in periodontal ligament (PDL), the osteogenic interface between tooth and bone, reveals there are three kinetically and/or morphometrically distinguishable cell types in the osteoblast histogenesis sequence: (a) self-perpetuating, less differentiated precursor cells (A type), (b) committed osteoprogenitor cells (A' type), and (c) preosteoblasts (C/D cells). The osteoblast (Ob) histogenesis sequence is $A \rightarrow A' \Rightarrow C \rightarrow D \rightarrow \text{Ob}$. The rate limiting step ($A' \Rightarrow C$) in differentiation of an osteoblast is associated with an increase in nuclear volume, immediately prior to the last proliferation event (each D cell divides and forms two Ob). This morphological manifestation of change in genomic expression (differentiation) has proven to be an effective tool for assessing inhibition of bone formation during spaceflight and simulated weightlessness (S-W). The nuclear volume assay of osteogenic activity and/or potential is applicable to all skeletal sites tested.

Research during the past year focused on further definition of the differentiation mechanism for osteoblast production at two skeletal sites: maxillary molar PDL and mandibular condyle (major growth site of the lower jaw bone). With respect to preosteoblast differentiation, the following principal questions were addressed:

(1) What is the effect of 17 days of simulated weightlessness (S-W), with an earlier more stressful version of the head down suspension model, in maxillary molar PDL and tibial primary spongiosa (PS)? (2) What is the effect on PDL of 14 days of S-W with the recent less stressful model? (3) What is the effect of 3 days of S-W (most recent version of head down suspension model) on bone cell kinetics of PDL? (4) What is the circadian rhythm of PDL preosteoblast cells following 3 days of simulated weightlessness? (5) What is the influence of 7 days of spaceflight on the osseous morphology and cell kinetics of osteogenic cells in the mandibular condyle of Spacelab (SL)-3 rats?

Experiments were conducted in rats using hind limb unloading to simulate the preceding conditions.

Accomplishments

(1) Seventeen days of S-W with more stressful model resulted in relatively fewer preosteoblasts (C/D cells) in both PDL and tibial PS.

(2) Fourteen days of S-W with the less stressful model failed to suppress preosteoblast numbers in PDL or tibial PS.

(3) Three days of S-W with the less stressful model was sufficient to produce a transient block in preosteoblast differentiation.

(4) Following 3 days of S-W, relative numbers of preosteoblasts were decreased at both 9 am and 9 pm.

(5) Mandibular condyles of SL-3 rats had significantly decreased cartilage volume, trabecular bone and number of osteoblasts.

(6) Bone lining cells within the metaphysis of the mandibular condyle from SL-3 rats had increased less differentiated (A+A') cells and decreased preosteoblasts (C+D).

(7) SL-3 mandibular condyles had a relatively increased cortical bone volume.

Significance of the Accomplishments

Finding #1. Physiological stress, as evidenced by failure to gain weight during the experimental period, is an important variable in S-W projects that must be carefully controlled.

Finding #2. Failure to suppress preosteoblast numbers long term (14 days) indicates that the latest version of the S-W model is not physiologically stressful and that the initial osteogenic depression seen after 3 days of S-W is a transient effect.

Finding #3. The timing of transient osteogenic response, after 3 days of simulated weightlessness, is consistent with cell kinetic estimates for suppressing bone formation by curtailing preosteoblast production (block in A'=>C).

Finding #4. The transient decrease in bone formation noted 3 days after initiation of simulated weightlessness is associated with

decreased numbers of preosteoblasts during both light and dark periods. These data suggest that decreased preosteoblast production is relatively uniform throughout the circadian cycle.

Finding #5. These results indicate that 7 days of spaceflight is sufficient to elicit morphological changes consistent with decreased skeletal growth. The significant decrease in numbers of osteoblasts is particularly important because it indicates the mandibular condyle is more sensitive to weightlessness than other trabecular bone surfaces, i.e. lumbar vertebrae (Wronski, et al., Am. J. Physiol. 252: R252-R255, 1987).

Finding #6. Relatively fewer preosteoblasts lining bone surfaces suggest a decreased rate of osteoblast differentiation. These data indicate at least a portion of the osteogenic deficit of spaceflight is secondary to an inhibition in osteoblast differentiation.

Finding #7. As the mandibular growth rate slows during spaceflight, the increased ratio of cortical to trabecular bone is due to cancellous compaction (filling in of cancellous bone to create compacta) in the condylar metaphysis.

Publications

Brezniak, N. and Roberts, W.E. Multiple Fluorochrome Histomorphometric Analysis of Rat Trabecular Bone (Abstract). Journal of Dental Research 65: 235, 1986.

Brezniak, N. and Roberts, W.E. Relative Incidence of Different Color Fluorochrome Labels at Four Weekly Intervals in Rat Trabecular Bone (Abstract). Anatomical Record 214: 16A, 1986.

Burk, D.T., Gonsalves, M.R., and Roberts, W.E. Vascularly Oriented Migration Patterns of Osteogenic Cells Along Bone Forming Surfaces of Rat Periodontal Ligament (PDL) (Abstract). Anatomical Record 214: 16A, 1986.

Crawford, L.B., Fielder, P.J., and Roberts, W.E. Morphometric Analysis of Periodontal Ligament (PDL) and Alveolar Bone of Unopposed Rat Molars (Abstract). Journal of Dental Research 65: 235, 1986.

Fielder, P.J., Morey, E.R., and Roberts, W.E. Osteoblast Histogenesis in Periodontal Ligament and Tibial Metaphysis During Simulated Weightlessness. Aviation, Space and Environmental Medicine 57: 1125-1130, 1986.

Roberts, W.E., Poon, L.C., and Smith, R.K. Development of Rigid Osseous Interface With Tricalcium Phosphate (TCP)-Coated Endosseous Implants (Abstract). Journal of Dental Research 65: 347, 1986.

Roberts, W.E., Poon, L.C., and Smith, R.K. Interface Histology of Rigid Endosseous Implants. Oral Implantology 12: 406-415, 1986.

Roberts, W.E., Rosenoer, L.M.L., and Weinstein, R.S. Nuclear Volume Analysis of Cells Lining Normal Human Trabecular Bone (Abstract). Journal of Bone and Mineral Research (Suppl.): 123, 1986.

Roberts, W.E., Weinreb, M.M., Gonsalves, M.R., and Morey, E.R. Nuclear Volume Assay of Osteogenic Potential in Ulna Primary Spongiosa of Rats Flown in Space for 18.5 Days (Abstract). Anatomical Record 214: 18A, 1986.

Rosenoer, L.M.L., Fielder, P.J., Morey, E.R., and Roberts, W.E. Osteogenic Index of Rat PDL on Forming and Resorbing Bone Surfaces (Abstract). Journal of Dental Research 65: 331, 1986.

MAMMALIAN GRAVITY RECEPTORS: STRUCTURE AND METABOLISM

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Description of Research

The goals of this research remain twofold: (a) to understand the functional organization of mammalian vestibular maculas (linear bioaccelerometers), and (b) to understand the role of calcium in their functioning. The current focus is on information processing by the maculas. That is, the accomplishments section will indicate that work with long series of sections and with computer-based reconstructions is revealing that maculas process information in parallel, as do retina and brain. They can be understood best in terms of an information network, which is a computer technology. Thus, emphasis must be expanded on the first of the two listed goals for the near future, because it is here that the greatest contribution can be made.

The research will capitalize upon unique computer facilities and technological expertise available at NASA's Ames Research Center. We shall continue to use serial sections of portions of maculas as a basis for our reconstructions. We already have every fifth section of a series of 580 sections photographed and in montage form. At first, representative examples of each kind of terminal field will be traced onto acetate and digitized into an image analyzer. The images will be assembled with the aid of a computer. This stage of the research will be used to produce the proper programs for conversion of our images into solids or transparencies, using an IRIS work station. Next, linkages between terminal fields will be illustrated, building up the network that processes information. This will require development of appropriate graphics because of the complexity of the data. Following these developmental stages, new sections will be cut for assembly using every (or every other) section for the reconstructions. This is necessary in order to locate all synapses. Concurrently, effort will be expended to utilize edge detection capabilities to automate capture of information from the electron microscope onto laser disk. The computer ability should be maximized to enable at least semiautomatic reconstruction of the digitized data. The reconstructions themselves will show "weightings" in the network, i.e., number and location of synapses, and number of linkages between individual terminals of the network. This will enable us to produce a dynamic model of a functioning network, based upon network theory.

Accomplishments

(1) Reconstructions based upon 160 sections of saccular macula demonstrated that there are three kinds of nerve terminal

patterns: (a) a U-type, which has a long, unmyelinated precalyceal segment and complex calyces which have numerous collaterals, both afferent and efferent in type; (b) an M/U-type, which has a short, unmyelinated precalyceal segment and a calyx with few or no collaterals; and (c) an M-type, in which the nerve is myelinated to the calyx which appears to lack collaterals.

(2) All three kinds of terminals are also present in the utricle, according to analysis of a new series of 580 serial sections.

(3) In all nerve patterns, type II hair cells are integrated into the neural circuitry of the type I cells. Type II cells usually end on more than one calyx (up to four, thus far). Thus, information flowing through type II hair cells is distributed.

(4) Certain type II hair cells synapse with a calyx, or unmyelinated nerve segment, and then help form clusters of type II cells. The type II hair cells interdigitate cell b orders but no gap or ribbon junctions have been observed. Such cell clustering may be a way of stopping information flow in specific directions, thus defining a local network (or circuit) consisting of several terminal fields.

(5) An M-type nerve terminal field was reconstructed in 3-dimensions. It contained five type I cells and eight type II cells. Six of the eight type II cells had their heads within the territory outlined by type I cells at the macular surface. This could not have been suspected on the basis of work with montages alone.

(6) A first, symbolic diagram of a weighted neural network was produced based on reconstructions made and in progress. This diagram demonstrates that all three kinds of nerves are linked in some of the circuits.

(7) One result of research with calcium precipitation methods for ultrastructural study of calcium distribution in maculas was that supramacular substance has been better defined. There are macromolecules in the substance indicating that it might be a weak gel. A "subcupular" space, postulated by several previous investigators, does not exist.

(8) Another result of this research is the finding that stereocilia are linked together by macromolecules; and the kinocilium is largely linked to adjacent stereocilia. The kinocilium has all the ultrastructural features of a motile cilium. These findings have implications for transduction. Macromolecules link kinocilia to surfaces of adjacent stereocilia. They also link stereocilium to stereocilium at sites along the stereociliary membrane where internal linkers connect actin to the surface membrane. Our results show, then, that there is a route from the internal motor driving kinociliary motility, the dynein arms, to the actin of the stereocilia.

Significance of the Accomplishments

(1) The first three accomplishments support the contention that type II hair cells of mammalian maculas lack an independent innervation.

(2) The first six findings relate to the observation that the maculas process information as an information network. This interpretation is new and reverses previous morphological results which indicated that hair cells passed on information to their nerve terminals serially. According to this previous concept, each kind of hair cell had its own line to the central nervous system. New results show instead that macular terminal fields process information in parallel. It should be added that the discrepancy between old and new interpretations is the result of our modern ability to do ultrastructural analysis of long series of sections.

(3) The sixth accomplishment is significant. It is the first attempt to display the macular circuit as a weighted neural network.

(4) Further advances in our knowledge of the weighting and connectivity of the neural network will be useful to computer theoreticians trying to design parallel processor computers which better mimic the brain.

(5) Accomplishments #7 and 8 resolve discrepancies in the literature regarding supramacular substance (endolymph?). The results negate the recent hypothesis that transduction involves specific connections between stereociliary apices.

Publications

Ross, M.D. Implications of Otoconial Changes in Microgravity. Physiologist 30(1, Suppl.): S90-S93, 1987.

Ross, M.D. and Donovan, K. Otoconia as Test Masses in Biological Accelerometers: What Can We Learn About Their Formation From Evolutionary Studies and From Work in Microgravity? Scanning Electron Microscopy IV: 1695-1704, 1986.

Ross, M.D., Komorowski, T., Donovan, K., and Pote, K.G. The Suprastructure of the Saccular Macula. Acta Otolaryngologica 103: 56-63, 1987.

Ross, M.D., Rogers, C., and Donovan, K. Innervation Patterns in Rat Saccular Macula: A Structural Basis for Complex Sensory Processing. Acta Otolaryngologica 102: 75-86, 1986.

Ross, M.D., Rogers, C.M., and Holt, G. 3-D Reconstruction of an M-type Sensory Field in Rat Utricular Macula (Abstract). Association for Research in Otolaryngology, p. 105, 1987.

EFFECTS OF MICROGRAVITY ON SEA URCHIN FERTILIZATION AND EARLY DEVELOPMENT

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Description of Research

Gravity has been a pervasive influence on all living systems, and there is convincing evidence to suggest that it alters fertilization and embryogenesis in several developmental systems. Notwithstanding the global importance of gravity on development, it has only been recently possible to begin to design experiments which might directly investigate the specific effects of this force. The goal of this research program is to explore and understand the effects of gravity on fertilization and early development using sea urchins as a model system. Sea urchin development has several advantages for this project, including the feasibility of maintaining and manipulating these cells during spaceflight, the high percentage of normal fertilization and early development, and the abundant knowledge about molecular, biochemical, and cellular events during embryogenesis, which permit detailed insights into the mechanisms by which gravity might interfere with development. Furthermore, skeletal calcium is deposited into the embryonic spicules within a day of fertilization, permitting studies of the effects of gravity on bone calcium deposition.

Accomplishments

(1) Clinostat experiments were performed to explore the effects of constant changes in the vector of gravitational force. At several rotational speeds ranging from 1/4 through 60 revolutions per minute, fertilization and early development occurred normally. These studies indicate that clinostat experimentation does not affect development in this system and serves as a foundation on which to design spaceflight experiments.

(2) The conditions necessary for spaceflight experiments were investigated, including the materials for holding the gametes prior to launch, the culture environment, the fixation protocols, and the parameters necessary to permit optimal development in a sealed environment.

(3) The time span for holding unfertilized eggs and mature sperm was determined, and several environmental parameters required to lengthen this span were investigated.

(4) Sperm kept between 0° C and 4° C retain their viability for over 24 hr: this is sufficient for preflight storage and use after launch.

(5) Unfertilized eggs are not viable for longer than 6 hr

under normal conditions. Conditions have been optimized to maintain unfertilized egg viability for between 14 to 18 hr by controlling the temperature at 15° C with oxygenation. This duration might permit a sufficient time for storing the gametes prior to launch, though other parameters are being investigated.

(6) Culture conditions for spaceflight experiments are being designed which might permit normal development for up to 5 days. At present, sealed plastic culture containers permit normal fertilization and development for 24 hr, if an oxygen bubble of one-third of the volume is included in the chamber.

Significance of the Accomplishments

The clinostat studies demonstrated the utility of this system for providing convincing conclusions quickly and inexpensively. With this foundation, spaceflight is now justified. The remaining accomplishments demonstrate the potential feasibility of this system for spaceflight studies. Many of the details necessary for pre-flight, in-flight and post-flight preparation and storage have been identified with likely solutions.

Publications

Cline, C.A. and Schatten, G. Microfilament Detection During Fertilization: Rhodaminy Fluorescence Microscopy. Gamete Research 14: 271-299, 1986.

Maul, G.G. and Schatten, G. Nuclear Lamins During Gametogenesis, Fertilization and Early Development. In: Nucleocytoplasmic Transport, pp. 123-134, 1986.

Schatten, G., Bestor, T., Schatten, H., Balczon, R., and Henson, J. Intracellular pH Shift Initiates Microtubule Assembly and Motility During Fertilization. Annals of the New York Academy of Sciences 466: 940-944, 1986.

Schatten, G. and Schatten, H. Cytoskeletal Organization During Fertilization in Mice and Sea Urchins. In: New Discoveries and Technologies in Developmental Biology, International Congress on Developmental Biology 10: 91-94, 1986.

Schatten, G. and Schatten, H. Motility and Cytoskeletal Organization During Fertilization in Sea Urchins and in Mice. Cell Motility 6: 163-175, 1986.

Schatten, H., Schatten, G., Mazia, D., Balczon, R., and Simerly, C. Behavior of Centrosomes During Fertilization and Cell Division in Mice and Sea Urchins. Proceedings of the National Academy of Sciences, USA 83: 105-109, 1986.

Schatten, G., Schatten, H., Paweletz, N., Spector, I., Simerly, C., and Petzelt, C. Latrunculin Prevents the Microfilament-mediated Processes of Fertilization and Cytokinesis. Experimental Cell Research 166: 191-208, 1986.

Schatten, G., Simerly, C. and Schatten, H. Microtubules in Mouse Oocytes During Fertilization. Annals of the New York Academy of Sciences 466: 948-952, 1986.

EFFECTS OF WEIGHTLESSNESS ON AURELIA EPHYRA DIFFERENTIATION AND STATOLITH SYNTHESIS

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Description of Research

The long-range goals of the research are (a) to discover the role(s) of gravity on the behavior and the development of Aurelia ephyrae and on their graviceptor structures and (b) to discover the effects of microgravity on ephyra and rhopalial development after short-term (Shuttle flight) and long-term (Space Station and Biosatellite) exposure to a microgravity environment.

Specific objectives are:

(1) To determine whether the microgravity of spaceflight will modify: the development of ephyrae from polyps; the development of the graviceptors of ephyrae; the formation or demineralization of statoliths of rhopalial; and the swimming/pulsing behavior of ephyrae.

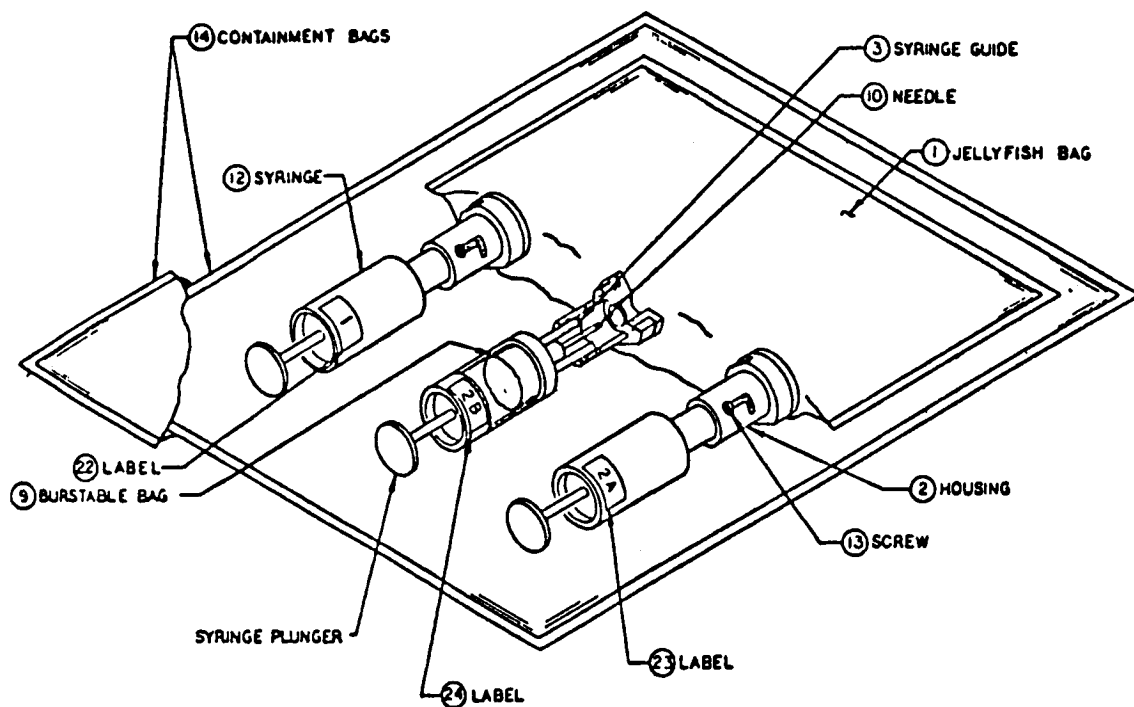
(2) To compare the features listed above in ephyrae which developed in space with those that developed on Earth and to discover the role that gravity plays in the development of ephyrae, their graviceptors and their behavior.

(3) To prepare the Jellyfish Experiment for the 7-day Shuttle flight Space Life Sciences (SLS)-1.

This year, the Jellyfish Experiment was manifested for Shuttle flight SLS-1. This experiment consists of three components which provide the opportunity to compare the effects of microgravity on polyps induced to metamorphose on Earth (Package A) with those induced to metamorphose in space (Package B). Swimming/pulsing behavior of ephyrae which have developed on Earth will be compared with ephyrae which will develop in space (Package C). In addition, ephyrae with developed statoliths will be flown and their rate of demineralization compared with ground-based controls. In order to implement the experiment for proposed flight conditions, more than 3000 jellyfish were tested using the Aurelia Metamorphosis Test System.

(1) Chemical Delivery System. A Chemical Delivery System for introduction of chemicals into plastic bags containing jellyfish was developed (in collaboration with Suzanne Davis, Karen Rossberg, and Chris Todd). After testing several types of plastic bags for their biocompatibility and testing various types of clamps to separate the chemicals from the animals, a syringe system attached to a 2 mil Kapak bag was conceptualized and developed (Figure 1). Biocompatibility of the parts of the Delivery System was determined by exposing the developing jellyfish to them. It was found that the materials used to make parts #7 and #8 were toxic to the jellyfish (see Figure 1). New

CHEMICAL DELIVERY SYSTEM FOR JELLYFISH EXPERIMENT



SEPTUM ASSEMBLY

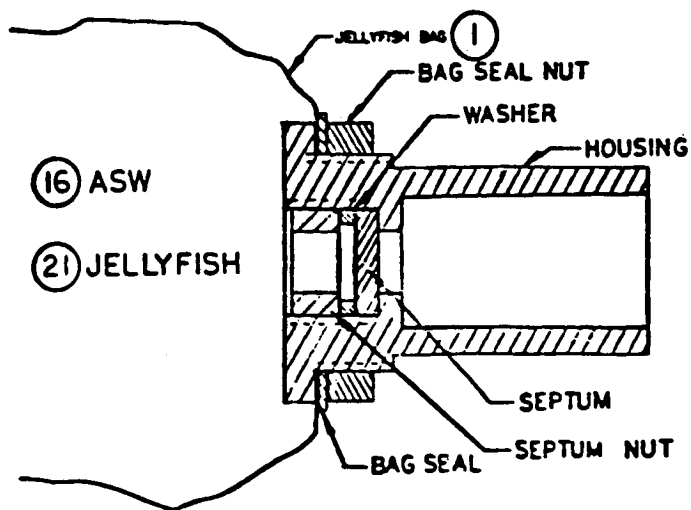


Figure 1. Chemical Delivery System developed for injection of chemicals into plastic bags containing jellyfish during spaceflight. This system will permit metamorphosis to be induced and the jellyfish to be fixed while in space.

materials were substituted and the Delivery System was assembled and tested. The chemicals to be used in the flight experiment, iodine and thyroxine, were added to the bags containing the jellyfish at the beginning of the experiments and the fixative (glutaraldehyde) was injected into some of the bags containing the jellyfish at the end of the experiments. Comparison of the developmental features of the ephyrae (morphology, statolith number, and pulsing/swimming behavior) which had developed in the Delivery System with controls revealed no significant differences. Comparison of fixation of rhopalia from ephyrae in the Delivery System with controls, using the transmission electron microscope, likewise revealed no significant differences.

(2) Air/artificial sea water (ASW)/animal ratio studies.

(a) Strobilation studies: Comparisons were made of polyps developing in various containers with various ratios of air to ASW to animals. Although ephyrae can develop in a low air environment, optimum development proceeds with a ratio of 100 parts ASW:50 air:100 polyps. The volume of ASW and air can be reduced one-fourth per 100 polyps and the quality of development of ephyrae is still very good.

(b) Pulsing ability: Tests were done of the pulsing ability of ephyrae which had developed in Falcon tissue culture flasks and of ephyrae which were merely maintained in these flasks for 5 days. Efforts were made to determine the lowest amount of air required for normal pulsing. After three separate tests, it was determined that 33 ephyrae will develop in the flasks with as little as 0.25 ml of air/65 ml of ASW, but 0.5 ml of air is needed for normal pulsing activity (determined by comparison with controls with a large amount of air). This small amount of air is not expected to impede photography of the ephyrae.

(3) Centrifugation. Centrifugation tests were done to determine whether the polyps and ephyrae can withstand g levels greater than one. Groups of polyps and ephyrae were centrifuged at 3 g and 5 g at Ames Research Center (with the assistance of Dr. D. Tonka). The ephyrae continued to swim immediately after centrifugation and polyps appeared normal when compared to non-centrifuged controls. In a separate experiment, groups of polyps were centrifuged at 9 g for 5 min. The test groups and controls were then induced to strobilate with iodine. The resulting ephyrae were compared with ephyrae from non-centrifuged polyps and were found to be normal as regards form, statolith number, and pulsing.

(4) Fixation: Glutaraldehyde and buffer stored for 9 days at 28°C were compared with refrigerated solutions in their ability to fix the jellyfish ephyrae. Several organisms from each group were embedded and sectioned following fixation and their tissues were compared using the transmission electron microscope. The quality of fixation of tissues using the stored fixatives was comparable to controls. Fixation of rhopalia of ephyrae following introduction of fixative to the animals using the Chemical Delivery System (see above) was also comparable to controls.

(5) Clones. A series of experiments were done to establish the continuing validity of cloning polyps for their ability to produce normal ephyrae. Clones were developed in 1985 and have been tested periodically since that time. Over a period of 18 months, the polyps from these clones have consistently given rise to a high level of normal ephyrae, as compared with organisms randomly selected from the cultures.

(6) Temperature Variation. Groups of polyps were exposed to various temperature treatments based on possible flight experiment scenarios. Some were given iodine at 28°C for 24 or 48 hr and then dropped to a temperature of 19°C for 24 or 48 hr and then raised again to 28°C. Some groups were kept at 19°C for 24 or 48 hr and then raised to 28°C for the remainder of the experiment (9 days). Controls were maintained at 19°C or 28°C for nine days. All of the groups produced ephyrae within nine days and all except the 19°C treatment group had pulsing, statolith numbers, and morphological development within a normal range. Only those organisms grown at 19°C for the duration of the experiment had increased arm abnormality and significant reduction of pulsing and statolith numbers.

Accomplishments

(1) Development of a Chemical Delivery System for injection of chemicals to the jellyfish in Kapak bags during flight. Chemicals, including the fixative glutaraldehyde can be safely delivered using this system.

(2) Determination was made of the optimum air/water/animal ratio for jellyfish containment in Kapak bags and in tissue culture flasks.

(3) Determination was made that polyps and ephyrae are tolerant of centrifugal forces of 3 g, 5 g and 9 g. Indeed, the jellyfish will metamorphose normally following centrifugation.

(4) Fixation of ephyrae with glutaraldehyde proceeds normally following storage of glutaraldehyde at 28°C for 9 days.

(5) Polyps can be induced to strobilate at 28°C, transferred to 19°C for 24 or 48 hr, then transferred back to 28°C without causing abnormality of ephyrae.

Significance of the Accomplishments

Research this year centered around the practical need to prepare the jellyfish for a flight experiment. Laboratory protocol for strobilation studies is very simple on Earth but is complicated by the space and crew-time limitations of the Shuttle and the microgravity environment. Therefore, it was necessary to develop a Chemical Delivery System to induce metamorphosis and fix jellyfish in space. This Delivery System is easy to use, delivers chemicals, accurately and can be applied to other small aquatic animals being used for a flight experiment. Considerable time and effort was also expended to test other parameters which could impact on the execution of the proposed experiment. In so doing, we learned new facts about the jellyfish and their developmental processes.

It was found that they can develop very well in Kapak bags and tissue culture flasks in which they tolerate less air than in glass containers. The jellyfish can withstand g forces of at least 9 g for 5 min and subsequently develop normally following metamorphosis. The organisms can also withstand temperature changes from 28 to 19 to 28°C during metamorphosis and produce normal ephyrae. The ephyrae do not develop normally, however, following 9-day exposure to 19°C, although they do produce ephyrae.

The jellyfish experiment will be used to determine whether the graviceptors form normally in space. If they do not develop normally, we will deduce that gravity is needed for normal development (having controlled for other factors) and that gravity plays an important role in the normal development of these structures on Earth. To determine whether the graviceptors formed in space are normal, we will examine them in detail, with various types of microscopy, and compare them with controls developed on Earth. An in-depth understanding of the mechanisms through which these organisms sense and respond to gravity is important in understanding how other organisms are affected by gravity and will illuminate the role(s) of gravity in the development of functioning of organisms on Earth as well as the effects of microgravity on biological organisms in space.

Publications

Spangenberg, D.B. Statolith Formation in Cnidaria: Effects of Cadmium on Aurelia Statoliths. Scanning Electron Microscopy IV: 1609-1618, 1986.

SKELETAL MUSCLE METABOLISM IN HYPOKINETIC RATS

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Description of Research

Research in this laboratory is aimed at identifying the mechanisms leading to the metabolic adaptations of muscle to lack of load-bearing (unloading), as may occur with prolonged bedrest or weightlessness (Figure 1). In particular, we are endeavoring to separate out the sequence of events leading to these adaptations especially related to increased protein degradation. Our work in the past year has led us to consider how the mechanism of unloading atrophy differs from denervation atrophy and how it relates to turnover of specific protein pools.

The thrust of our research over the past year has gone in two major directions. First, we have continued investigating carbohydrate metabolism and glucose transport. Together with data for 3-6 days, we have been able to follow the time course for changes in these parameters over six days of unloading. Related to these studies have been the changes in insulin responsiveness or sensitivity, and the effects of recovery on these processes.

Our other major emphasis has concerned changes in protein metabolism in both unloaded and denervated soleus muscle. Experiments have focused on the potential sites of proteolysis (i.e., lysosome vs. cytoplasm), the responsiveness of protein synthesis and degradation to insulin, and the possible role of Ca^{2+} in accelerated proteolysis.

In the course of comparing these models of muscle atrophy, we have also considered whether denervation can override the effects of unloading. A few related experiments have considered amino acid transport and amino acid levels in the muscle.

Accomplishments

1. Protein metabolism (Figure 2)

(a) The sensitivity of protein synthesis and degradation to insulin increases in the unloaded soleus when the muscle atrophies.

(b) Soleus muscle which is both denervated and unloaded shows a resistance of in vitro protein synthesis to insulin rather than the increased sensitivity due to unloading.

(c) Denervated, unloaded soleus shows faster in vitro proteolysis in the absence or presence of insulin with no apparent difference in response to insulin.

(d) Lysosomotropic agents, which diminish lysosomal

EFFECTS OF UNLOADING AND DORSIFLEXION IMMOBILIZATION ON RAT HIND LIMB MUSCLES

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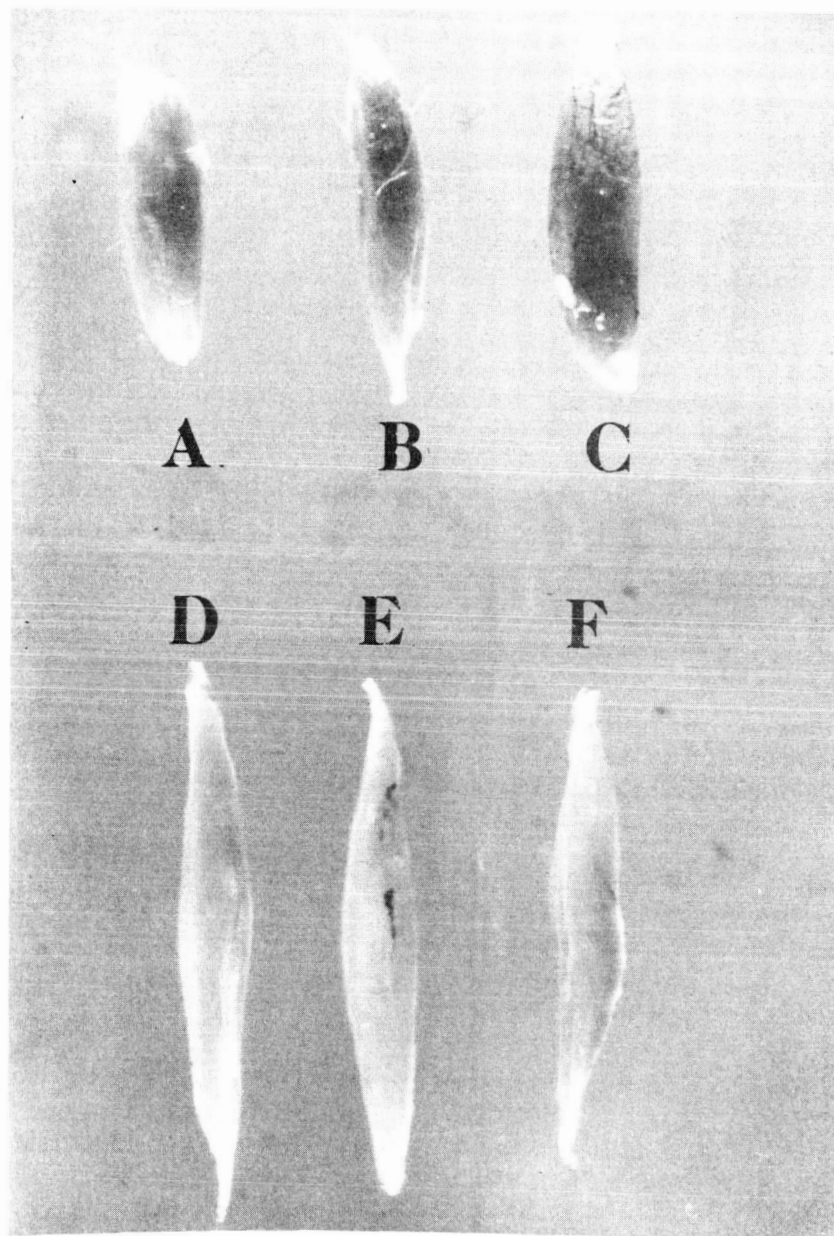


Figure 1. Effects of unloading and dorsiflexion immobilization on rat hind limb muscles. Soleus (A-C) and extensor digitorum longus (D-F) muscles from weight bearing (A,D) or 6-day tail-cast unloaded rats (100 g) having one limb freely-moving (B,E) or immobilized in dorsiflexion (C,F) to stretch the soleus and shorten the extensor digitorum longus. Unloading atrophied the soleus (B) while stretch caused hypertrophy (C). Shortening atrophied the extensor digitorum longus (F).

function, reduce the accelerated in vitro proteolysis of denervated but not of unloaded soleus muscle.

(e) Mersalyl (inhibitor of non-lysosomal calcium activated protease) abolishes the accelerated in vitro proteolysis of unloaded muscle but only has a partial effect in denervated muscle. Abolishing endogenous release but not exogenous uptake of calcium reduces proteolysis in unloaded but not control muscle.

2. Amino acid metabolism

(a) Uptake of amino acid shows resistance to insulin in denervated, unloaded soleus but not in soleus unloaded only.

(b) Denervation and unloading produce similar effects on fresh tissue amino acids.

3. Carbohydrate metabolism

(a) The activity ratio for glycogen phosphorylase fell within 4 hr after unloading, followed by a sharp drop in glycogen synthetase.

(b) At 3-6 days unloading, insulin sensitivity increased for glucose uptake and metabolism in the soleus.

(c) Basal (in vitro) glucose transport fell within 4 hrs of unloading, was still lower after 24 hrs but returned to normal by 3 days.

(d) Diabetic, 3-day unloaded soleus showed lower basal glucose uptake than diabetic, weight-bearing soleus.

(e) Reloading leads to an initial fall followed by a marked rise in glycogen accompanied by a greatly elevated uptake of glucose. Glycogen enzyme activity ratios are reversed within 1 hr. Increased insulin sensitivity disappears by 24 hr of reloading.

Significance of the Accomplishments

Insulin Sensitivity

Finding #1 agrees with our prior conclusion that atrophy by unloading leads to increased insulin sensitivity due to greater binding capacity per muscle mass. In contrast, atrophy by denervation does not lead to greater binding capacity but may instead cause insulin resistance. Increased insulin sensitivity in the unloaded soleus was even apparent in vivo (Finding #3) although these results were indirect.

Lysosomal vs. cytosolic proteolysis

Preservation of the insulin receptor in unloading atrophy suggests little or no role of accelerated lysosomal proteolysis, as supported by our in vitro (Findings #1D). Instead the calcium-activated protease may be important in unloading atrophy, as well as to some extent in denervation atrophy, with endogenous but not exogenous calcium seemingly the more critical source (Finding #1E). Conceptually, the protease may be localized closer to this endogenous source (sarcomplasmic reticulum) in the unloaded than the normal muscle.

PROPOSED MODEL OF DIFFERENCES BETWEEN INSULIN RECEPTOR TURNOVER IN UNLOADED AND DENERVATED ATROPHYING MUSCLE

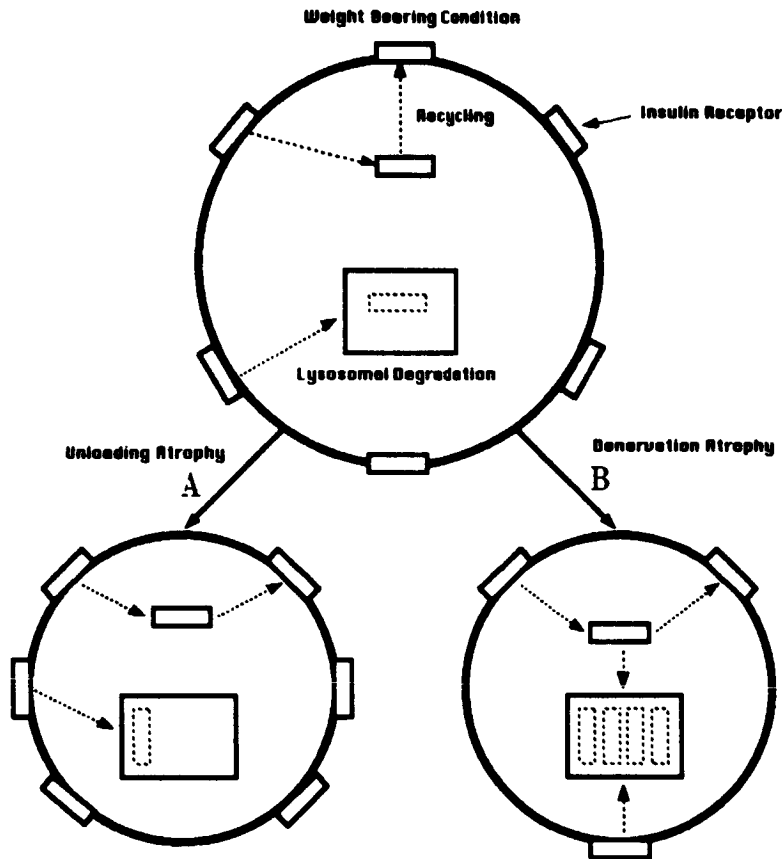


Figure 2. The insulin receptor can be recycled or degraded. Atrophy caused by unloading shows no net change in this process, because of no increased role of lysosomal proteolysis, so that binding capacity can be preserved (A). Atrophy due to cutting the nerve supply may lead to loss of receptors due to involvement of lysosomal proteolysis in denervation atrophy (B).

Time course of carbohydrate response

Our data support the following sequence of events (Finding #3A). Within the first 4 hrs of unloading, glycogen begins to accumulate due to a fall in the phosphorylase. Elevated glycogen in turn inhibits the synthase leading to a gradual decline in glycogen during 1 to 6 days of unloading. This fall in the synthase seems large enough to avoid being offset by increased insulin sensitivity (Finding #3B). In contrast, glucose transport is very responsive to changes in insulin sensitivity. Recovery of changes in carbohydrate metabolism whether acute (4 to 24 hr) or longer term (1 to 3 days) occurs rapidly and the overshoot in some instances is indicative of overcompensation by the muscle (Finding #3E). Similarly, marathon runners can superload their leg muscles with glycogen.

In general, the findings show that denervation overrides responses to unloading.

Publications

Fagan, J.M., Satarug, S., Cook, P., and Tischler, M.E. Rat Muscle Protein Turnover and Redox State in Progressive Diabetes. Life Sciences 40: 783-790, 1987.

Fagan, J.M. and Tischler, M.E. Reduction-oxidation State and Protein Degradation in Skeletal Muscle of Fasted and Refed Rats. Journal of Nutrition 116: 2028-2033, 1986.

Fagan, J.M. and Tischler, M.E. Reduction-oxidation State and Protein Degradation in Skeletal Muscles of Growing Rats. Growth 50: 139-146, 1986.

Henriksen, E.J., Tischler, M.E., and Johnson, D.G. Increased Response to Insulin of Glucose Metabolism in the 6-day Unloaded Rat Soleus Muscle. Journal of Biological Chemistry 261: 10707-10712, 1986.

Jaspers, S.R. and Tischler, M.E. Role of Glucocorticoids in the Response of Rat Leg Muscles to Reduced Activity. Muscle Nerve 9: 554-561, 1986.

EFFECTS OF SIMULATED MICROGRAVITY ON MAMMALIAN DEVELOPMENT AND EXPRESSION OF CALCIUM BINDING PROTEINS

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Description of Research

The long-range goal of our research is to assess the effects of altered gravitational environments on mammalian development and differentiation.

Our research has focused on examining the effects of a simulated microgravity environment on the development and differentiation of germ cells and early embryos. We previously showed that mouse oocytes rotated on a clinostat exhibited anomalies of the meiotic maturation process. Oocytes rotated at 100 rpm in an axis perpendicular to the gravity vector (the experimental rotation axis) were inhibited in the rate with which they achieved the metaphase II stage of meiosis, whereas oocytes rotated in the control orientation were not inhibited. In subsequent series of experiments, ova were rotated in the presence of sperm, thereby addressing the question of whether or not fertilization was sensitive to the effects of simulated microgravity. No alterations in the efficiency of fertilization were noted. Although the zygotes appeared normal, as judged by gross morphological criteria, the developmental potential of such rotated zygotes needs to be investigated. We have recently extended these studies to include examination of very early embryos. A recent modification of the experimental system involves embedding the embryos in low melting point agarose in order to immobilize them at the axis of rotation. This eliminates the effects of extraneous movements of the cells during rotation in a liquid suspension. Finally, we have begun to examine the use of sensitive molecular markers of normal development such that our experimental system as well as cells flown in space can be evaluated for subtle biochemical changes at the level of the single cell.

Accomplishments

(1) Sub-cellular events in mammalian cells may be affected by simulated microgravity. That is, reorientation of mammalian oocytes relative to the gravity vector, through the use of a clinostat, affects meiotic maturation. Oocytes rotated at 100 rpm in the experimental axis revealed an inhibition of achieving metaphase II of meiosis, whereas oocytes rotated in the control orientation did not. This may reflect inhibition of normal chromosome orientation and movement.

(2) No abnormalities in the appearance of fertilized ova or in the efficiency of fertilization have been observed in ova which rotated at 100 rpm at the time of fertilization. In these experiments, ova which had undergone meiotic maturation in vivo were placed with capacitated sperm in the clinostat rotation system. The ova were rotated at 100 rpm (the speed at which meiotic abnormalities had been observed) for 8 hr and were examined for the presence of pronuclei and for any morphological abnormalities. Although the ova appeared normal and fertilization rates were similar between the experimental and control systems, it will be of interest to evaluate more fully the developmental potential of such rotated ova.

(3) A modification of the culture system has been incorporated in our recent studies on the effect of simulated microgravity on early embryogenesis. In these experiments, embryos are embedded on low melting point agarose and immobilized at the center of the axis of rotation. With these conditions and rotation at 100 rpm, development up to the blastocyst stage has been achieved. These embryos will now be removed from the culture system and allowed to implant in the uteri of foster mothers in order to assess normal development.

(4) An effort has been initiated to develop sensitive molecular markers for normal development. This will enhance the ability to analyze experiments using the clinostat and, most importantly, will greatly enhance the potential analysis of space-flown tissues. The first molecular markers we are considering are the genes for the cellular stress proteins (also called heat shock proteins), using detection methods at the level of RNA and protein.

Significance of the Accomplishments

Finding #1. The observation of an effect on a division process of cells under conditions of reorientation relative to the gravity vector is significant at several levels. First, an inhibition of normal chromosome disjunction would affect the developmental potential of the ova. Second, these observations are of particular interest because of the observations of other investigators on the effects on mitotic divisions in cells which have been flown in space.

Finding #2. The observation of apparently normal fertilization under clinostat rotation suggests that formation of the male and female pronuclei is not sensitive to reorientation relative to the gravity vector.

Finding #3. Our use of the cells in the immobilized situation has considerably strengthened the arguments in favor of using the clinostat as a model for simulating microgravity. That is, we are able to eliminate extraneous cell movement as a factor in interpreting our results.

Finding #4. Sensitive indicators of normal development and differentiation are badly needed for assessing the effects of

altered gravitational environments on cells. Our studies on the expression of the cellular stress protein genes will be very useful for analyzing both ground-based and flight studies.

SPECIAL ACTIVITIES

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SPACE BIOLOGY RESEARCH ASSOCIATES' PROGRAM

NASA's Space Biology program provides a unique opportunity to train individuals to conduct biological research in outer space and to continue relevant ground-based research. To maximize the potential for Space Biology as an emerging discipline, there is a need to develop a cadre of scientists interested in working in this area. This grant was developed to train this cadre of biologists by offering Research Associate Awards to young scientists. These grants provide opportunities to work on projects directly related to Space Biology. The research is conducted in laboratories that provide the necessary facilities and a suitable research environment. It is anticipated that these scientists will develop research careers in the newly evolving discipline of gravitational biology since it is rapidly growing and its future will reflect the quality and training of its scientific personnel.

The program began on June 1, 1980 with funding to support several Research Associates each year. To date, 53 awards have been made. There have been 33 awardees, of whom 20 have received a second year of funding. On April 4, 1987, the NASA Review Panel will meet to fund 8 more scientists. These scientists come from many different disciplines including: zoology, developmental biology, botany, and physiology (animal and plant). They have been assigned to laboratories at the following institutions: University of California at Berkeley, Irvine, and Davis; Stanford University; University of Texas at Austin and Dallas; University of Houston; Texas A&M; University of Michigan; Wayne State University; Washington University; Indiana University; University of Louisville; Cleveland Clinic; National Institute of Health and National Institute of Mental Health; University of Pittsburgh; University of Pennsylvania; Rockefeller University; University of Washington; Michigan State University; Duke University; Tufts University; Princeton University; Yale University; and Dartmouth College. In June 1980 there were 19 laboratories participating. Presently (March 1987), there are 48 laboratories in the program.

The scientists who have completed this program have accepted positions in colleges and universities, with research laboratories and with NASA. Dr. Jay Buckey is a co-investigator and project manager for Dr. Gunnar Blomqvist's Spacelab-4 project; Dr. John Garavelli is working in the Extraterrestrial Research Division at NASA-Ames; Dr. Steven Black is working with Dr. Kenneth Souza at NASA-Ames; Dr. Mark Cooper is working with Dr. Emily Morey-Holton at NASA-Ames; and Dr. Dewey Meyers was the Science and Curriculum Coordinator in the Space Life Sciences Training Program at Kennedy Space Center. Drs. Kerr, Meyers, Slocum and Steffen have received research grants from NASA. Drs. Blair, Meyers, Robinson, Szilagyi and Torigoe have also submitted proposals that are presently being reviewed. Several other Research Associates plan to submit proposals in the near future.

In addition many of the Research Associates have been asked to participate in NASA panels, national workshops and national meetings. There have been 87 publications in refereed journals and as many abstracts of papers presented at national and international meetings.

Each year, in the fall, the Research Associates are requested to attend the annual meeting of the American Society for Gravitational and Space Biology (ASGSB). Prior to the formation of the Society, the Research Associates used to meet and present papers at an annual AIBS/NASA meeting. With the formation of the Society, the meeting was held in conjunction with the American Physiological Society/International Union of Physiological Sciences (APS/IUPS) Commission on Gravitational Physiology. In 1986, the ASGSB meeting was not integrated with other societies; it may be in the future that the Society will meet periodically with other Societies. In any event, the Research Associates have and will continue to be an integrated part in that meeting. All of the current Research Associates and many of the former Research Associates are members of ASGSB and present papers together with senior colleagues. Research Associates have been well represented in presenting papers and posters at the annual meetings of ASGSB. Thirteen Research Associates were present at this year's meeting and participated fully in the program. The Research Associates are also encouraged to participate in other national meetings in their own disciplines.

At the completion of their award period, they are required to submit a final report. These reports are on file in the Project Director and Scientific Advisor's office (Dr. X.J. Musacchia, University of Louisville).

Research Associate Awardees

As stated previously, this program has provided awards for thirty-three Research Associates. They are listed below (alphabetically): names, award terms (in parentheses after their name), host laboratory, and current location:

Dr. Michael Binder (1/1/83 - 12/30/83) worked on "Congenital Heart Malformations and Situs Inversus" in Dr. W.M. Layton, Jr.'s laboratory at Dartmouth Medical School. He is now on a research fellowship in the Pathology Department at Brown University, Providence, Rhode Island.

Dr. Thomas Bjorkman (10/1/86 - 9/30/87) is working on "The Mechanism of Gravity Sensing in Plants" in Dr. Robert Cleland's laboratory at The University of Washington, Seattle, Washington.

Dr. Steven Black (7/1/82 - 6/30/84) worked on "Determination by Gravitational and Centrifugal Force of the Amphibian Dorsal-ventral Axis" in Dr. Raymond Keller's laboratory at the University of California, Berkeley. He is continuing research with Dr. Keller and is also working with Dr. Kenneth Souza at

NASA-Ames Research Center, Moffett Field, California.

Dr. Harry Blair (7/1/84 - 6/30/86) worked on "Cellular Mechanisms of Bone Degradation" in Dr. Steven Teitelbaum's laboratory at The Jewish Hospital/Washington University Medical Center, St. Louis, Missouri. He is continuing to work in Dr. Teitelbaum's laboratory funded by a NIH Physician Scientist Training Grant.

Dr. Thomas Brock (8/1/86 - 7/30/87) is working on "Comparison of Changes in Protein Synthesis Induced by Gravity Auxin Treatment in Pulvini and Coleoptiles of Oat (Avena sativa L.) in Dr. Peter Kaufman's laboratory at The University of Michigan, Ann Arbor, Michigan.

Dr. Jay Buckey, Jr. (7/1/82 - 6/30/84) worked on "2-D Echocardiography as an Accurate Mean for Measuring Left Ventricular Volume and Central Venous Pressure during Zero-gravity" in Dr. C. Gunnar Blomqvist's laboratory at the University of Texas Health Sciences Center, Dallas. At the present time he is the project manager for the cardiovascular experiment scheduled on Spacelab-4 and a Research Assistant Professor/Instructor in Clinical Medicine at the University of Texas Health Science Center, Dallas, Texas.

Dr. George H. Burrows (7/1/81 - 6/30/83) worked on "Studies of Synaptogenesis" in Dr. Marshall Nirenberg's laboratory at N.I.H., Bethesda, Maryland. He is now on the staff of the National Heart, Lung, and Blood Institute, Bethesda, Maryland.

Dr. Denis Clohisy (7/1/86 - 6/30/87) is working on "Mechanisms of Osteoclast Precursor Differentiation" in Dr. Steven Teitelbaum's laboratory at The Jewish Hospital/Washington University Medical Center, St. Louis, Missouri.

Dr. Mark Cooper (1/1/85 - 12/30/86) worked on "Osteoporosis of Weightlessness and the Electrophysiology of Bone" in Dr. John Miller's laboratory at The University of California at Berkeley, California. He is a visiting Assistant Professor in the Biophysics Department at Berkeley as well as doing some work for Dr. Emily Morey-Holton at NASA-Ames Research Center, Moffett Field, California.

Dr. Mark Desrosiers (7/1/86 - 6/30/87) is working on "A Search for Voltage-gating of Plant Hormone Transport Channels" in Dr. Robert Bandurski's laboratory at Michigan State University, East Lansing, Michigan.

Dr. John S. Garavelli (1/1/82 - 4/30/82) worked on "Chemical Characterization of Volatile Products of Algal Cell Cultures" in Dr. Franklin Fong's laboratory at Texas A&M University. He is now working for the Extraterrestrial Research Division at NASA-Ames Research Center, Moffett Field, California.

Dr. John Gaynor (1/1/81 - 12/30/82) worked on "Purification and

Characterization of Amyloplasts from Pisum sativum" in Dr. Arthur Galston's laboratory at Yale University. He is now an Assistant Professor and Henry Rutgers' Scholar in the Botany Department at Rutgers' University, Newark, New Jersey.

Dr. Steven Glotzbach (1/1/84 - 12/30/84) worked on "Neurophysiological Studies of Circadian Rhythm Control Mechanisms" with Dr. H. Craig Heller at Stanford University and Dr. Charles A. Fuller at the University of California, Riverside. He is continuing to work in Dr. Heller's laboratory funded by a NIH-NIRA, Palo Alto, California.

Dr. Cheryl Gould (7/1/84 - 8/30/85) worked on "Effect of Weightlessness on Various Immunological Functions using a Murine Simulated Space Flight Model" in Dr. Gerald Sonnenfeld's laboratory at The University of Louisville, Louisville, Kentucky. She is now developing a Tissue Banking Program for the Community Blood Center, Dayton, Ohio.

Dr. Martha Gray (7/1/86 - 6/30/87) is working on "The Correlation of Applied Strain Distributions to the Location of New Bone Formation: A Rigorous Mechanical Analysis of an in vivo Bone Preparation" in Dr. Clinton Rubin's laboratory at Tufts University School of Veterinary Medicine, North Grafton, Massachusetts.

Dr. Marcia Harrison (7/1/83 - 8/30/85) worked on "Participation of Ethylene in Two Modes of Gravitropism of Shoots" with Dr. Barbara Pickard at Washington University, St. Louis. She is now an Assistant Professor in the Biology Department at Marshall University in Huntington, West Virginia.

Dr. Gary Jahns (1/1/83 - 4/30/84) worked on "Interactions of Light and Gravity on the Growth, Orientation, and Lignin Biosynthesis in Mung Beans" in Dr. Joe Cowles' laboratory at the University of Houston. He is continuing to work with Dr. Cowles, Houston, Texas.

Dr. Timothy Jones (1/1/81 - 12/30/82) worked on "The Effects of Hypergravic Fields on Brainstem Auditory-evoked Potentials" in Dr. John Horowitz' laboratory at the University of California, Davis. He is now an Assistant Professor at the University of Nebraska, Lincoln, Nebraska.

Dr. Thomas Kerr (1/1/83 - 12/30/84) worked on "Cellular Localization of Na^+ , K^+ -ATPase in the Mammalian Vestibular System"; the first year in Dr. Muriel Ross's laboratory at the University of Michigan and the second year in Dr. Dennis Drescher's laboratory at Wayne State University. He is now an Assistant Professor at Wayne State University, Detroit, Michigan.

Dr. Douglas Kligman (7/1/82 - 6/30/84) worked on "The Role of Neurite Extension Factor Nerve and Muscle Tissue Response to Stress or Injury" in Dr. David Jacobowitz' laboratory at the

National Institute of Mental Health, Bethesda, Maryland. He is now on the staff at NIMH, Bethesda, Maryland.

Dr. Konrad Kuzmanoff (7/1/83 - 7/30/85) worked on "Isolation and Identification of B-glucan Synthetase: A Potential Biochemical Regulator of Gravistimulated Differential Cell Wall Loosening" in Dr. Peter Ray's laboratory at Stanford University. He is now a Research Associate working with Dr. Craig Beattie at the University of Illinois at Chicago, Illinois.

Dr. Michael Matilsky (1/1/81 - 12/30/82) worked on "Gravity Perception in the Algal Coenocyte Caulerpa prolifera" in Dr. William Jacobs' laboratory at Princeton University. He is now working with the International Genetic Science Partnership in Talpiot, Jerusalem, Israel.

Dr. Dewey Meyers (7/1/81 - 6/30/83) worked on "Response, Adaptation and Gravitational Perception in a Parthenogenic Freshwater Microcrustacean, Daphnia galeata mendotae" in Dr. Allan Brown's laboratory at the University of Pennsylvania. He was the Science and Curriculum Coordinator in the Space Life Sciences Training Program at Kennedy Space Center, Florida. Recently, he became an Adjunct Associate Professor at West Virginia School of Osteopathic Medicine, Lewisburg, West Virginia.

Dr. Dean Murakami (1/1/85 - 12/30/86) worked on "Influences of the Hyperdynamic Environment on the Development of the Visual System in the Rat" in Dr. Charles Fuller's laboratory at The University of California at Davis. He is continuing to work with Dr. Fuller at The University of California, Davis, California.

Dr. Mary Musgrave (6/1/86 - 5/30/87) is working on "Studies of Respiratory Metabolism" in Dr. Boyd Strain's laboratory at Duke University, Durham, North Carolina.

Dr. Gary Radice (7/1/81 - 6/30/83) worked on "Control of Gravity-Sensing Mechanism in Amphibian Eggs" in Dr. George Malacinski's laboratory at Indiana University. He is continuing to work with Dr. Malacinski, Bloomington, Indiana.

Dr. Farrell R. Robinson, Jr. (7/1/84 - 6/30/86) worked on "Sensory Motor Properties of the Uvula and Nodulus" in Dr. David Tomko's laboratory at The University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. He is now working as a Research Associate with Dr. Albert Fuchs in the Physiology Department of the University of Washington School of Medicine in Seattle, Washington.

Dr. Bruce Serlin (7/1/84 - 6/30/85) worked on "Differential Wall Growth in Gravistimulated Corn Roots: Its Timing and Regulation" in Dr. Stanley Roux's laboratory at the University of Texas at Austin. He is now an Assistant Professor at DePauw University, Greencastle, Indiana.

Dr. Robert Slocum (1/1/81 - 12/30/83) worked on "Studies on the Localization and Functional Role of Calcium in Gravistimulated Plant Organs"; the first year in Dr. Stanley Roux's laboratory at the University of Texas at Austin and the second year in Dr. Arthur Galston's laboratory at Yale University. He is now an Assistant Professor at Williams College, Williamstown, Massachusetts.

Dr. J. Henry Slone (7/1/85 - 6/30/86) is working on "Characterization of the Protein Responsible for the Lateral Transport of Auxin during Gravitropism of Pea Shoots and Determination Whether Phosphorylation Participates in Gravitropic Activation" in Dr. Barbara Pickard's laboratory at Washington University in St. Louis, Missouri.

Dr. Joseph Steffen (7/1/81 - 6/30/83) worked on "Glucocorticoid Receptor Levels in Hindlimb Skeletal Muscles and Diaphragm During Prolonged (2 Week) Antiorthostatic Hypokinesia and Recovery" in Dr. X.J. Musacchia's laboratory at the University of Louisville. He is now an Assistant Professor at The University of Louisville, Louisville, Kentucky.

Dr. Julianna Szilagyi (7/1/81 - 12/30/81) worked on "Progressive Hemodynamic Changes in Simulated Weightlessness" in Dr. Carlos Ferrario's laboratory at the Cleveland Clinic. She is now an Assistant Professor at the University of Houston, Houston, Texas.

Dr. Yasuhiro Torigoe (1/1/84 - 12/30/85) is working on "Anatomical Correlated Underlying Vestibulo-autonomic Outflow to the Gut" with Dr. Robert H. I. Blanks at the University of California, Irvine. He is continuing to work with Dr. Blanks at the University of California, Irvine, California.

THE MECHANISM OF GRAVITY SENSING IN PLANTS

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Description of Research

The goal of this research is to discern events associated with the initial steps of gravity sensing. Many responses of sensing cells should be discernible by electrophysiological means. In order to assess the role of components such as electrogenic proton pump, ion channels, intracellular calcium, and action potentials in signal transduction, a tissue is needed where the sensing cells can be identified in the live tissue by observation and where they are on the surface.

Using such a system, the objective is to test whether a depolarization like that observed in sensing cells of Lepidium occurs and whether gravistimulation induces a transient increase in the cytoplasmic calcium activity. The latter is to test the oft-proposed hypothesis that calcium acts as a second messenger in signal transduction.

Accomplishments

(1) Decapped Golden Cross Bantam maize roots begin to recover gravisensitivity after 23 hr and responsiveness increases until full recovery at 40 hr after decapping.

(2) The root cap begins to regenerate after about 36 hr.

(3) The presentation time of gravisensitive roots lacking root caps (28 hr after decapping) is 9 min. The presentation time of control roots is 6 min.

(4) Roots, where superficial mucilage and the apical half of the root cap are removed, respond the same as control roots.

Significance of the Accomplishments

Decapped roots regain gravisensitivity before the cap regenerates. The apical cells (formerly the quiescent center) are the gravisensitive ones and contain sedimenting amyloplasts according to Barlow (J. Exp. Bot. 25: 1137, 1974). These cells satisfy the requirements for direct electrophysiological observation.

The presentation time gives a time frame within which gravity sensing must occur. The similarity of presentation times suggest that gravity sensing occurs by the same mechanism in both cell types.

Removal of most of the calcium-rich mucilage does not inhibit the graviresponse, suggesting that it does not play a central role in graviresponsiveness.

Publications

Bjorkman, T. and Leopold, A.C. Electric Current Changes Associated With Gravity Sensing in Corn Roots (Abstract). Plant Physiology 80(4, Suppl.): 26, 1986.

BIOCHEMICAL MECHANISMS OF BONE DEGRADATION

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Description of Research

Skeletal loss in space, like any other form of osteoporosis, reflects a relative imbalance of the activities of cells which resorb (degrade) or form bone. Under these circumstances osteoclasts, which are the major bone resorbing cells, degrade more bone than is simultaneously formed by osteoblasts. Consequently, prevention of a microgravity-induced bone loss may theoretically be accomplished by (a) stimulating bone formation or (b) inhibiting bone resorption. Clearly, however, such an approach would require a fundamental understanding of the mechanisms by which cells form or degrade bone. Thus, our objective has been to study one of the basic functions of bone cells, namely the means by which osteoclasts degrade bone. The cellular complexity of the experimental models previously used to examine the resorptive process precluded one from distinguishing, with certainty, the activities of osteoclasts from those of other cells resident in the systems. Hence, the crux of our NASA-supported research was development of an experimental model of bone resorption containing virtually pure populations of osteoclasts. Under these circumstances, it would be possible to attribute a particular biological phenomenon to osteoclasts and to evaluate the capacity of these cells to degrade the various components of bone.

Accomplishments

We are pleased to report that during the period of NASA research funding we have achieved this goal, and we have pushed substantially beyond. Briefly, the osteoclast produces an acidic microenvironment at the bone-attachment interface using an electrogenic $F_1F_0H^+$ pump; cellular pH is maintained at near neutrality by Cl^-/HCO_3^- exchange at the anti-attachment surface (Figure 1). The resultant low pH solubilizes bone mineral. The osteoclast secretes into the secondary lysosome-like attachment site an acid hydrolase of approximately 28 kD, which degrades collagen below pH 6, probably utilizing a mannose-6-phosphate receptor system to transport the enzyme to the degradation site (Figure 1). Specific accomplishments from which this model is derived include (see also "Publications" for detailed results):

(1) For the first time, an in vitro model of bone resorption based on virtually homogeneous (98%+) populations of bona fide osteoclasts has been produced. These cells retain the ruffled membranes, acid phosphatase productions, and osteoclast-specific antigens in culture, features which

MECHANISM OF BONE DEGRADATION BY OSTEOCLASTS

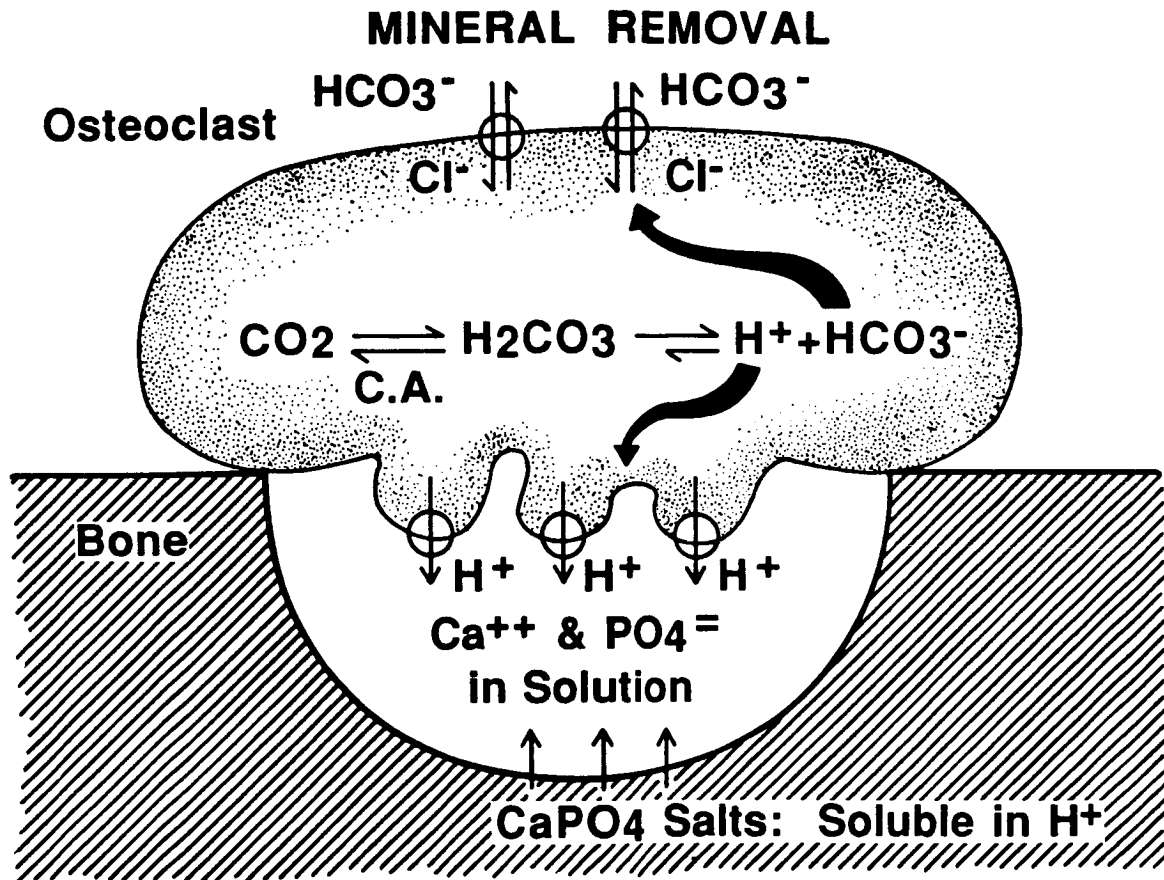


Figure 1. Illustrations of model by which osteoclasts resorb both the inorganic (above) and organic (opposite) components of bone. Above. An acidic microenvironment produced at the site of attachment to bone by a hydrogen ion pump solubilizes bone mineral. Facing page. Osteoclasts secrete an acid collagenase which degrades collagen at low pH.

characterize the same cells in vivo.

(2) The ability of osteoclasts to resorb both the organic and inorganic components of bone has been documented quantitatively, a new development.

(3) Degradation of bone collagen to fragments less than 10,000 MW has been shown.

(4) It has been demonstrated that osteoclast activity is confined to the cellular attachment site, since labeled collagen fragments added to supernatants of actively resorbing osteoclasts on bone were recovered intact.

(5) Enzyme activity in osteoclast lysates, which results in degradation of bone collagen at pH 4.2, has been demonstrated. This is the first direct evidence that osteoclasts degrade bone by acidifying the attachment site to remove mineral and secreting acidic proteases to degrade collagen.

More recently, we have shown:

(1) Osteoclast degradation is blocked when its ability to acidify the attachment site is blocked.

(2) An acidic protease has been purified from osteoclasts which degrades collagen at low pH.

(3) Mannose-6-phosphate receptors, which transport acidic proteases to lysosomes, have also been documented on the osteoclast surface.

(4) Presence of an electrogenic F_1F_0 H^+ pump has been demonstrated in the osteoclastic ruffled membrane.

(5) Osteoclasts maintain internal pH, at least in part, by Cl^-/HCO_3^- exchange at the anti-attachment surface.

Publications

Blair, H.C., Dean, D., Muniz, O.E., Jeffrey, J.J., Teitelbaum, S.L., Woessner, J.F., and Howell, D.S. Biochemical and Immunological Evidence for Collagenase-mediated Matrix Erosion by Hypertrophic Chondrocytes (Abstract). Clinical Research 34: 731A, 1986.

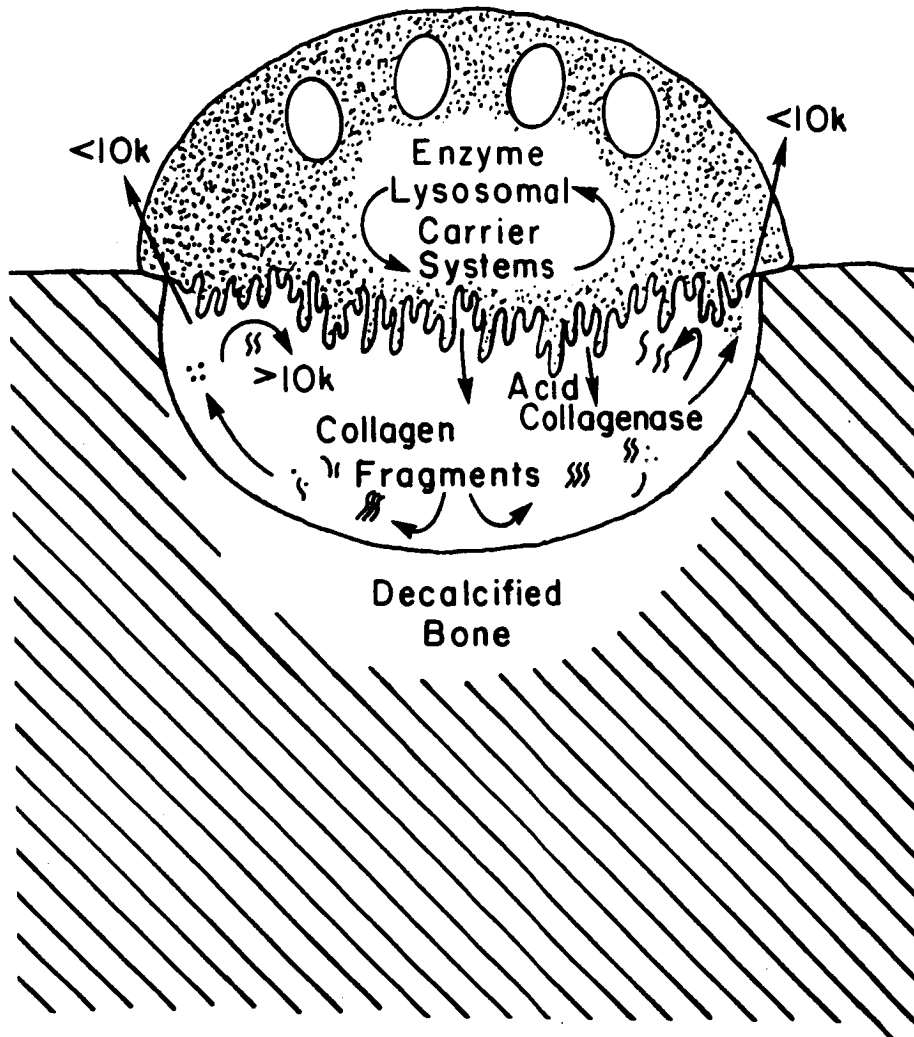
Blair, H. and Ghandur, L. Macrophage-mediated Bone Resorption Occurs in an Acidic Environment. Calcified Tissue International 37: 547-550, 1985.

Blair, H.C., Jeffrey, J.J., and Teitelbaum, S.L. Osteoclasts Resorb Bone Collagen by Acidic Proteolysis (Abstract). Journal of Bone and Mineral Research 1: S53, 1986.

Blair, H.C., Kahn, A.J., Crouch, E.C., Jeffrey, J.J., and Teitelbaum, S.L. Isolated Osteoclasts Resorb the Organic and Inorganic Components of Bone. Journal of Cell Biology 102: 1164-1172, 1986.

Blair, H., Kahn, A.J., Jeffrey, J.J., and Teitelbaum, S.L. Isolated Osteoclasts Resorb the Organic and Inorganic Components of Bone (Abstract). Calcified Tissue International 37: S10, 1985.

ORGANIC MATRIX DEGRADATION



Blair, H.C., Kahn, A.J., Jeffrey, J.J., and Teitelbaum, S.L.
Lysates of Pure Osteoclast Populations Degrade Bone Collagen at
pH 4.8 (Abstract). Physiologist 28: 311, 1985.

Blair, H.C., Kahn, A.J., Jeffrey, J.J., and Teitelbaum, S.L.
Osteoclasts Resorb Bone Collagen by an Acidic Protease
(Abstract). Journal of Cell Biology 101: 97A, 1985.

Blair, H.C., Teitelbaum, S.L., Ehlich, L.S., and Jeffrey, J.J.
Collagenase Production By Smooth Muscle. Correlation of
Immunoreactive With Functional Enzyme in the Myometrium. Journal
of Cell Physiology 129: 111-123, 1986.

THE MECHANISM OF THE GRAVIRESPONSE OF THE LEAF-SHEATH PULVINUS

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Description of Research

This research program is directed at understanding the cellular basis of growth in plants, using the oat pulvinus as a model system. Both gravity and exogenous hormones induce growth in this system. Our approach involves comparing their effects and examining their interactions.

The main goals of this year's research were: (a) to identify when the oat pulvinus first becomes competent to show a graviresponser and when it is maximally responsive and (b) to characterize the kinetics of the graviresponser. This work forms the foundation for subsequent projects dealing with the role of endogenous hormones in directing the graviresponser, the possible changes in sensitivity of the pulvinus, and the kinetics of cellular responses to exogenous hormones.

Accomplishments

(1) The oat pulvinus (p-1 locus) goes through three distinct developmental stages. During the earliest stage, the pulvinus increases in length, width, and volume and is largely unable to show a graviresponser. The intermediate stage is characterized by a cessation of pulvinus growth and the onset of competency in terms of the graviresponser. Finally, the pulvinus resumes growth, most notably in width, and gradually loses the capacity to show a graviresponser.

(2) The intermediate stage is correlated with the release and exponential growth phase of the supertending internode. The final stage coincides with cessation of internodal elongation and the release of the inflorescence internode above.

(3) Although the pulvinus stops enlarging during the intermediate stage, it continues to develop with respect to how it responds to gravity. Most notably, the maximum steady state response rate (deg/hr) gradually increases as the pulvinus develops. On the other hand, the lag time to initial response remains relatively constant throughout the intermediate stage.

(4) We have developed a protocol for studying localized, rapid effects of applied hormones on cell expansion. Preliminary results indicate that applied auxin will induce a response comparable to that induced by gravity, and that gravity alters the responsiveness of tissue to applied auxin.

Significance of the Accomplishments

These results suggest that the cereal pulvinus can be viewed as a

discrete unit designed specifically to perceive and respond to changes in orientation with respect to gravity. They also identify critical features of the pulvinus graviresponse system and indicate directions for future projects.

The transition between the earliest stage and the intermediate stage is relatively short. During this transition period the capacity of the pulvinus to show a graviresponse rapidly develops. This presents a system that is well suited for identifying the cellular features that must change to produce graviresponsiveness of the system. Similarly, the final stage of development offers a system to identify the features that change to lead to loss of the graviresponse.

The intermediate stage differs significantly from early and late stages in terms of graviresponse kinetics. The identification of this stage and its characteristics provides the starting point for future work on the mechanism of the graviresponse.

The protocol for applying hormones appears to be very promising. Several preliminary experiments have indicated that this can be a powerful approach to studying the role of hormones in gravitropism and the effect of gravity and hormones on cellular aspects of growth.

MECHANISMS OF OSTEOCLAST PRECURSOR DIFFERENTIATION

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Description of Research

Skeletal bone loss in space, like all forms of osteoporosis, reflects a relative predominance of bone resorbing over bone forming cells. This project studies the development of bone resorbing cells (osteoclasts). Since osteoclasts are incapable of cell division, differentiation from their monocyte precursors will determine the rate of osteoclast formation. Vitamin D is the most potent physiological osteoclastogenic agent known. This investigation specifically aims to define the mechanism through which vitamin D promotes osteoclastogenesis. Our approach has been to characterize vitamin D's effect on mononuclear phagocyte differentiation, as these cells are known to be precursors to the mature bone resorbing osteoclast.

During the past year this research has addressed these questions: (a) Does vitamin D influence the formation of monocyte colonies? (b) At what stage in monocyte development does vitamin D exert its colony-inhibiting influence? (c) Is expression of the mannose-fucose cell membrane receptor altered with monocytic differentiation and does vitamin D modify its expression? (d) Is vitamin D's effect on the mannose-fucose cell membrane receptor specific regulation or does it represent a global effect on all cell membrane receptors?

Accomplishments

(1) Vitamin D inhibits monocyte colony formation in a dose-dependent, metabolite-specific fashion and the specificity of this inhibition reflects the relative affinity of each metabolite for the vitamin D receptor.

(2) Vitamin D's inhibition of colony formation is mediated exclusively through the developmentally mature adherent monocyte precursor.

(3) Monocyte cell membrane expression of the mannose-fucose receptor increases with monocytic differentiation and treatment with vitamin D accelerates this receptor's appearance and enhances its expression.

(4) Regulation of mannose-fucose receptor expression by both differentiation and vitamin D is specific in that the cell membrane expression of IgG2a receptors is unaltered.

Significance of the Accomplishments

(1) Vitamin D inhibits monocyte formation in a receptor-mediated fashion. Since decreased proliferation of any cell is usually associated with differentiation, this suggests that vitamin D promotes differentiation of these osteoclast precursors.

(2) Vitamin D's influence on monocyte colony formation is targeted at the more mature adherent cells. There are two distinct monocytic developmental stages prior to adherence where vitamin D exhibits no biological effect. These data suggest that the monocyte, which is likely to participate in osteoclast formation, is the developmentally mature adherent monocyte.

(3) Monocytic differentiation increases expression of cell membrane mannose-fucose receptors and vitamin D enhances this expression. This is definitive evidence that vitamin D promotes monocytic differentiation and is consistent with the hypothesis that vitamin D's osteoclastogenic effects may be mediated through enhanced differentiation of these osteoclast precursors.

(4) Neither monocytic differentiation nor vitamin D alters expression of IgG2a cell number receptors. This demonstrates that mannose-fucose receptor regulation is a specific marker of both monocytic differentiation and is specifically enhanced by vitamin D.

ELECTROPHYSIOLOGY OF BONE AND THE OSTEOPOROSIS OF WEIGHTLESSNESS

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Description of Research

The goal of this research is to determine the mechanisms underlying the growth reactions of osteoporotic bone in response to electrical stimuli. There are two major reasons for examining these responses. First, endogenous electrical currents, known as streaming potentials, are generated within bone when it is mechanically loaded. These electrical currents are viewed as a possible trophic mechanism for stimulating bone growth in response to mechanical loading of the skeleton. Secondly, several laboratories have reported that externally applied electric fields can counteract or reverse disuse osteoporosis in immobilized limbs of animal models. Identical electrical stimuli have been used clinically to promote the healing of human non-union bone fractures.

As a means of understanding the cellular sites of stimulation in these systems, the magnitude and time course of field-induced shifts of membrane potential were calculated for various cell types within bone using electrotonic cable theory. These calculations were aimed at determining the efficacy of specific electrical waveforms in stimulating the electrophysiology of osteogenic tissues. In addition, the magnitude and waveforms of electrical stimuli found to be effective in stimulating bone growth in avian limbs were experimentally measured.

Accomplishments

(1) Time-dependent cable theory was developed to quantitatively describe the hyperpolarization and depolarizations induced in cell membranes by pulsed electric and electromagnetic fields.

(2) We demonstrated that for autonomic nerves in bone, electric fields of magnitude E induce maximum shifts in membrane potential given by $-E\lambda$, where λ is the electrical length constant of the nerve. Charging of the cell membrane follows an exponential time course dictated by the RC time constant (τ) of the cell membrane. Similar electrical behavior holds for ensembles of osteoblasts coupled by gap junctions. Individual, uncoupled osteoblasts show smaller perturbations to the applied field, but can be polarized at much faster rates, reaching steady-state polarization within 0.01τ .

(3) Measurements of electric field magnitudes and waveforms induced in bone of stimuli used by Dr. Clinton Rubin (Tufts University) to counteract disuse osteoporosis in avian limbs were made.

(4) We established that gap junctional coupling occurs between osteobalsts in culture using injected fluorescent dyes (in collaboration with Dr. Emily Holton, NASA Ames Research Center).

Significance of the Accomplishments

The electrotonic cable theory developed in this study provides a framework to explain how externally applied electric and electromagnetic fields couple to the electrophysiology of bone cells. In addition, the theory generates guidelines for designing electrical pulse magnitudes and pulse durations to maximally stimulate cells within bone. These results have additional application in improving waveforms used in transcutaneous nerve and muscle stimulation.

The ability to stimulate autonomic nerves as well as osteoblasts within bone using electrical fields offers unique opportunities to modify local tissue physiology. Blood circulation and vascular permeability are under the control of autonomic nerves in many tissues. The local excitation of these nerves may enhance the delivery of endogenous growth hormones or systemically applied drugs to affected osteoporotic tissues. In characterizing the electric field strengths and waveforms which have been found to be effective in treating osteoporosis, the work of this study contributes to the development of such techniques.

The finding that osteoblasts retain gap junctional coupling in culture has implications in basic bone physiology. In situ, intercellular communication via such junctions may be important in nutrient transfer and growth regulation. In addition, gap junctional coupling between osteoblasts will cause cells to react as an electrical syncytium to applied electric stimuli.

Publications

Cooper, M.S. Electrical Cable Theory, Transmembrane Ion Fluxes, and the Motile Responses of Tissue Cells to External Electric Fields. In: Proceedings of the IEEE Conference, Engineering in Medicine and Biology, pp. 387-390, 1985.

Cooper, M.S. Electrophysiology of Bone and the Osteoporosis of Disuse. Physiologist 28: 311, 1985.

VOLTAGE-GATING OF PLANT HORMONE TRANSPORT CHANNELS

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Description of Research

The objective of this research is to understand how the perception of gravity by plants is transduced into a growth response. We postulate that the transduction mechanism involves an interaction between the plant's internal electrical gradients and transport of the plant growth hormone, indole-3-acetic acid (IAA), within the plant. Changes in a plant's orientation with respect to gravity are followed by a realignment of the plant's internal bioelectric field. This realignment in the bioelectric field opens and/or closes voltage-gated hormone-transporting channels between the plant's vascular tissue and the surrounding cortical tissues leading to accumulation of the hormone on the lower side of the shoot. This results in lateral asymmetric growth until the plant's axis is again vertical.

We are testing this theory by measuring how both the plant growth rate and the hormone concentration levels in different parts of the plant are altered by the application of external electric potentials. With the plant growing in a 1 g environment, the endogenous bioelectric fields are probably optimized for maximum growth and any disruption should result in growth inhibition. Plant growth is nominally related to the presence of the plant growth hormone IAA in the actively growing tissues.

Accomplishments

We developed an apparatus for applying a steady electric potential along the length of the shoot of 4-day old corn seedlings and used it to determine the electrical parameters that would influence the shoot's growth without causing damage to the plants. The effect of the electric potential upon the growth rate of the plants was found to depend on two factors: (1) the polarity of the potential, and (2) the magnitude of the potential. At low electrical potentials (less than a 0.6 mV drop over one 10 μ m length cell), making the tip of the shoot positive relative to the base decreased the shoot's growth rate by 90%, whereas the reverse polarity had little or no effect upon the shoot's growth rate. The magnitude of the electric current passing through the plants did not depend upon its polarity.

At electric potentials greater than a 1.9 mV drop per cell, shoot growth was inhibited with both polarities, but always the tip-positive polarity plants were inhibited to a greater extent than the tip-negative plants. Also at the higher potentials, the

onset of the growth inhibition occurred sooner with increasing voltages.

Significance of the Accomplishments

Plant growth can be regulated by the application of external electric potentials.

Normal plant growth may be possible in a microgravity environment by the use of small electric fields of the correct polarity to substitute for gravity.

THE CORRELATION OF APPLIED STRAIN DISTRIBUTION TO THE LOCATION OF NEW BONE FORMATION: A RIGOROUS MECHANICAL ANALYSIS OF AN IN VIVO PREPARATION

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Description of Research

A major physiological consequence of spaceflight is a decrease in bone mass. Presumably, this osteopenia is induced by the reduction in skeletal loading which occurs in the microgravity environment. However, the basic factors responsible for initiating and controlling bone remodeling processes are not well understood. The objective of this research is to better define the relationship between changes in the local mechanical environment of the skeleton and the local remodeling reaction this might stimulate. This knowledge will provide valuable insight into the mechanisms responsible for the control of bone mass, and may define means of preventing microgravity-induced bone loss.

Using an in vivo ulna preparation, this laboratory has previously found that a remodeling response occurs (effected by both new bone growth and bone resorption), and is dependent on applied load magnitude. This preparation provides the unique opportunity to rigorously test the hypothesis that there is a correlation between a change in mechanical environment and remodeling response. Our research is directed at testing this hypothesis by quantitatively determining the load-induced strain distribution so that this distribution can be compared directly to the local changes in bone architecture.

Accomplishments

The strains induced by load magnitudes spanning those used in the in vivo remodeling experiments have been measured using three 3-element rosette strain gauges positioned about the circumference of the midshaft. From these measurements we have determined the following:

(1) The longitudinal strain magnitude increases non-linearly with applied load magnitude.

(2) The bone undergoes bending, with the caudal surface subjected to tensile strains and the cranial and ventral surfaces subjected to compressive strains.

(3) By assuming that strain gradients are linear, the strain distribution was calculated for the midshaft cross section. The neutral axis (line of zero strain) consistently passed through the caudal-ventral corner and the caudal end of the cranial surface.

Significance of the Accomplishments

Finding #1. The nonlinear relationship between strain and applied load is most likely the result of complicated bone geometry leading to nonlinear structural deformation. This suggests that the remodeling activity elsewhere in the ulna may be very different than at the midshaft because the strain environment must vary considerably throughout the ulna. We are currently pursuing this by developing a finite element model of the ulnar preparation in collaboration with Dr. Tom Brown at the University of Iowa.

Finding #2 and #3. The knowledge of the strain distribution at the midshaft provides an opportunity to compare local strain patterns to regions of bone remodeling seen in the in vivo experiments. While there is intracortical porosis at the caudal-ventral corner where there is zero strain, there is no porosis in the cranial cortex where there is also a region of zero strain. Regions of bone formation do not bear any obvious relationship to longitudinal strain magnitude or strain gradient. These results suggest that the remodeling is not a direct function of longitudinal strain magnitude. We are currently considering the role of shear and transverse strain. We believe this avian ulna model, together with our ability to rigorously determine the strain environment, represents a significant and unique opportunity to examine the various hypotheses regarding the influence of mechanical factors on bone remodeling.

THE EFFECT OF GRAVITATIONAL FIELDS ON CENTRAL NERVOUS SYSTEM FUNCTION

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Description of Research

The object of this research program is to examine changes in central nervous system function in response to varying gravitational environments. It has been shown that altered gravitational fields affect many physiological variables such as prominent fluid shifts, lipid and protein metabolism changes, changes in plasma volume, temperature regulation, sleep, and circadian rhythms. Each of these physiological changes requires an adaptive change in the central hypothalamic mechanisms regulating these functions. This portion of the research program examined changes in metabolism of hypothalamic nuclei of adult rats exposed to either the microgravity condition of spaceflight or a 2 g hyperdynamic field. Metabolism in the nuclei were revealed with the metabolic stain cytochrome oxidase (CyOX).

Another objective in this research program was to examine the effects of altered gravitational fields on the development of the central nervous system. Animals have evolved and developed within the constant gravitational environment of the Earth. However, it is not known how dependent such developmental mechanisms are on a normal gravitational environment. This program has focused on two neural systems: (a) the retina because of its well characterized developmental patterns, and (b) the suprachiasmatic nucleus (SCN), a nucleus critical in the neural control of circadian rhythms. Circadian rhythms are known to be affected by gravitational changes.

Accomplishments

(1) The paraventricular nuclei and supraoptic nuclei exhibit increased metabolism following exposure to a 2 g hypergravitational field via centrifugation relative to 1 g controls.

(2) The paraventricular nuclei and supraoptic nuclei exhibit decreased metabolism following 10 days of spaceflight on Spacelab-3.

(3) The innerplexiform layer (IPL) of the retina in 28-day old rats conceived and raised in a 2 g field exhibits a significant decrease in thickness relative to controls.

(4) During normal development the rat SCN exhibits a central to peripheral temporal gradient of maturation in metabolic activity.

(5) Rats exposed to a 2 g field during prenatal development exhibit a posterior to anterior temporal gradient of maturation

in metabolic activity.

Significance of the Accomplishments

Findings #1 and #2: The paraventricular (PVN) and supraoptic (SON) nuclei are important in the neural control of fluid balance. The changes in metabolism following exposure to spaceflight or hypergravity exhibit a corresponding change in the metabolism of the PVN and SON. These metabolic changes may reflect the prominent fluid shifts to a new steady-state commonly observed under altered gravitational conditions. These preliminary results suggest that the neural mechanisms involved in the fluid homeostasis function normally in response to changes in gravity.

Finding #3: These results suggest that chronic exposure to hyperdynamic fields during development leads to significant neural deficits in the visual system. It will be important to quantify the possible physiological and behavioral deficits suggested by the morphological changes.

Findings #4 and #5: These studies demonstrate that exposure to hyperdynamic fields during development leads to an altered pattern of metabolic development in the SCN. Future studies are necessary to determine the possible deficits in circadian function due to the change in SCN development.

Publications

Miller, J.D., McMillen, B.A., Murakami, D.M., McConnaughey, M.M., Williams, H.L., and Fuller, C.A. Effects of Weightlessness on Neurotransmitter Receptors in Skeletal Brain Areas. Physiologist 28(6, Suppl.): S203-S204, 1985.

Miller, J.D., McMillen, B.A., Murakami, D.M., McConnaughey, M.M., Williams, H.L., and Fuller, C.A. Effects of Weightlessness on Neurotransmitter Receptors in Selected Brain Areas (Abstract). Physiologist 28: 377, 1985.

Murakami, D.M. and Fuller, C.A. Changes in Rat Brain Metabolism Following Exposure to Hyperdynamic Environment (Abstract). Physiologist 28: 313, 1985.

Murakami, D.M. and Fuller, C.A. Changes in Rat Retina and Brain Metabolism Following Exposure to Hyperdynamic Environments. Physiologist 28(6, Suppl.): S205-S206, 1985.

Murakami, D.M., Miller, J.D., and Fuller, C.A. Changes in Functional Metabolism in the Rat Central Nervous System Following Spaceflight. Physiologist 28(6, Suppl.): S143-S144, 1985.

Murakami, D.M., Miller, J.D., and Fuller, C.A. Changes in Functional Metabolism in the Rat Central Nervous System Following Spaceflight (Abstract). Physiologist 28: 377, 1985.

STUDIES ON THE RESPIRATORY METABOLISM OF PLANTS UNDER SPACEFLIGHT CONDITIONS

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Description of Research

This research is directed toward understanding and counteracting the effects of microgravity on respiratory metabolism in plants. Convective air movement, both on large and small scales, is a driving force of gas exchange in plants and is lacking in spaceflight conditions. Ultrastructural studies of material from short-term flight experiments showed that mitochondria may develop aberrantly, with features similar to those developed under anaerobic conditions. This suggests that the failure of plants to grow well for extended periods in space might be a consequence of impaired metabolism resulting from insufficient gas exchange.

During the past year this research has focused on a possible countermeasure to the limitations on gas exchange imposed by microgravity. Growing plants at reduced pressure would speed up diffusive processes by increasing the mean free path and could possibly compensate for the absence of convection in microgravity. Because of the structural and material supply advantages which would be afforded by low pressure plant growth areas, they have been proposed as an element in a CELSS (Controlled Ecological Life Support System). With the current concept of employing robots to tend the plants which would be grown for food and atmosphere regeneration in such a closed system, the need to stay within a pressure range tolerated by man is obviated. Little is known about the growth of plants at low pressure. This research has investigated the effects of reduced pressure (1/5 atmosphere) on plant growth and development, with special attention to respiratory metabolism.

Accomplishments

Mungbean seedlings were successfully germinated hypobarically (0.2 atm). Growth at low pressure was better than that at ambient pressure whether the flow through gas was air or 100% oxygen. When 100% oxygen was the flow-through gas, respiratory pathways developed as in ambient controls. When air was the flow-through gas, low pressure seedlings were somewhat more energetic than ambient pressure controls, with a higher total respiratory rate and lower percentage of cyanide-resistant (alternative) respiration in the mitochondria.

With regard to the presence or absence of the alternative respiratory pathway, a breeding and selection program made

the development of genetic lines of plants which differ possible. This pathway has been shown to make large differences in the cumulative response of plants to non-Earth normal metabolic gas ratios. These lines are being tested for their differential responses to the proposed low pressure growth regime which can allow for the most suitable plant varieties to be selected.

The use of cabin air at low pressure would have a potential drawback at the end of the plant's life cycle because while low partial pressures of oxygen stimulate vegetative development, seed set is inhibited. In ambient pressure experiments in the Duke University Phytotron, this inhibitory effect of low oxygen (5%) was overcome by simultaneous CO₂ enrichment (1000 ppm) in wheat and Brassica campestris.

Significance of the Accomplishments

(1) The finding that respiratory metabolism and growth are normal (or enhanced) at low pressure even when air is the flow-through gas encourages the idea that hypobaric growth of plants could be a useful technology for space applications. Contrary to previous suggestions that oxygen would have to be supplemented for successful growth at low pressure, these results show that cabin air could be circulated in a low pressure flow-through plant growth area with no detrimental effects on respiratory metabolism.

(2) The finding that seedlings grow better at low pressure than at ambient suggests that they are diffusion limited, not by availability of oxygen, but by dissipation of contaminants (such as ethylene). Experiments on the Shuttle which require higher purge rates than presently available could make use of this low pressure technology to achieve an adequate purge.

(3) Relative amounts of main chain and alternative respiration changed at low pressure only when the partial pressure of oxygen was different from that at ambient conditions. Since this balance plays a role in determining total plant carbon budgets, it will be important to study the response of plants which differ with regard to the amount of alternative respiration. These lines have been found in wheat, pea and Brassica campestris and are being screened in the environmental test chambers.

(4) At ambient pressure, sterility of plants grown at 5% oxygen can be overcome by CO₂ enrichment (1000 ppm). This suggests that at low pressure, the use of cabin air (which is enriched in CO₂) may overcome the sterility which would otherwise be expected at low oxygen partial pressures. Experiments to determine the conditions necessary for seed-to-seed cycling of plants under low pressure are currently underway.

CENTRAL VESTIBULAR PROCESSING IN THE CONTROL OF EYE AND HEAD MOVEMENTS

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Description of Research

This research is directed at understanding how vestibular and visual information influences eye and head movement control. We performed five experiments:

(1) We recorded from single vestibular neurons in the inferior olive. These olivary cells project and powerfully influence the vestibulocerebellum, but vestibular olivary responses have not been previously described.

(2) We recorded vertical eye movements in cats during pitch rotation around the interaural axis oriented horizontally (normal pitch) and around the interaural axis oriented vertically (on-side pitch). Normal pitch cause, the cat's head to change its orientation to gravity whereas on-side pitch does not. Comparison of the vertical vestibuloocular reflex (VVOR) movements in these two pitch conditions allowed us to evaluate how gravity-sensitive mechanisms contribute to VVOR movements.

(3) We recorded from single neurons in the vestibular nuclei of cats during trained voluntary head movements. These neurons have been characterized during passive rotation but not during normal voluntary head movements.

(4) We collaborated with Drs. J. Baker and B. Peterson of the Physiology Department of the Northwestern University Medical School on a comparison of cat neck muscle activation during voluntary and reflex head movements. We recorded EMGs from several neck muscles during trained voluntary head movements and during the same movements elicited reflexively by rotating the cat.

(5) In collaboration with Dr. M. Ariel of the Department of Behavioral Neuroscience at the University of Pittsburgh, we characterized the effect on vestibular and optokinetic eye movements by applying picrotoxin and APB to the retina. Previous work has established that picrotoxin makes directionally selective (DS) retinal ganglion cells respond to visual movement in all directions and APB reduces or eliminates the responses of D S retinal ganglion cells. Behavioral effects of these drugs have not been previously described but will provide strong evidence for or against the hypothesis that DS retinal ganglion cells have a direct influence on optokinetic eye movements. We

tested cats before and after ablation of visual cortical areas to determine how visual cortex influences the responses to these drugs.

Accomplishments

(1) Vestibular neurons in the inferior olive detect head movement in a particular direction. But, unlike other central vestibular neurons, they do not carry parametric information about the movement's velocity or acceleration.

(2) Gravity-sensitive mechanisms improve the gain and symmetry of the VVOR, particularly at low frequencies.

(3) About 1/3 of the neurons we recorded in the vestibular nuclei (mostly from the medial vestibular nucleus) integrate neck proprioceptive information with vestibular input.

(4) A voluntary head movement is accomplished by contracting neck muscles in a different pattern from that evident during the same movement made reflexively.

(5) Application of picrotoxin to the retina causes spontaneous nystagmus with the slow phase away from the treated eye. This nystagmus adds a constant velocity component to vestibular and optokinetic reflex eye movements. APB sharply reduces or eliminates optokinetic nystagmus without affecting vestibularly elicited eye movements. These effects are evident even after the visual areas of the cortex have been removed.

Significance of the Accomplishments

Finding #1. A vestibular olivary neuron supplies the cerebellum with information about a head rotation by firing only when particular properties are present and not, as other sensory cells do, by firing at different rates to different stimuli. This specialized sensory response, together with the inferior olive's strictly organized projection to the cerebellum and the powerful effect of olivary cells on cerebellar activity, indicates that a wide range of vestibular stimuli with a particular attribute (i.e., the correct direction) radically alters activity of a small region of the vestibulocerebellum. Future research will focus on characterizing the organization of different vestibular olivary inputs to the cerebellum and how these inputs influence cerebellar output and the related motor adaptation.

Finding #2. Gravity-sensitive sensory mechanisms, i.e., otoliths and/or gravity sensitivity in the semicircular canals, make the VVOR more compensatory. This sensitivity to gravity means that pitch in micro-g will cause inappropriate retinal slip and therefore visual-vestibular mismatch. Such mismatch is probably an adequate stimulus for motion sickness. Gravity sensitivity in the VVOR may therefore explain why, in micro-g, pitching the head with the eyes open causes faster and more severe onset of motion

sickness than head yaw or pitch with the eyes closed (Lackner and Graybiel, Aviat. Space, and Environ. Med. 55: 513, 1984).

Finding #3. During voluntary movement, which is the most frequent cause of vestibular stimulation in a normal animal, neck input often influences vestibular processing at an early stage. This information will focus future research on how this early integration of neck and vestibular information contributes to movement control.

Finding #4. The brain uses a different combination of neck muscles to cause the same movement, depending on whether the movement is voluntary or reflexive. Future research will identify the voluntary and reflex pathways and determine why they activate different patterns to cause the same movement.

Finding #5. The effects of applying picrotoxin and APB to the retina is the first direct behavioral evidence that DS retinal ganglion cells can exert a clear, strong influence on optokinetic and vestibularly elicited eye movements. The pathway through which DS retinal ganglion cells drive eye muscles does not go through the cortex.

Publications

Ariel, M and Robinson, F. Intravitreal Picrotoxin Affects Optokinetic and Vestibular Eye Movements via Subcortical Pathway in Cat (Abstract). Society for Neuroscience Abstracts 12: 1086, 1986.

Keshner, E., Baker, J., Banovetz, J., Peterson, B., Wickland, C., Robinson, F., and Tomko, D. Neck Muscles Demonstrate Preferential Activation During Voluntary and Reflex Head Movements in the Cat (Abstract). Society for Neuroscience Abstracts 12: 684, 1986.

Robinson, F., Fraser, M., and Tomko, D. Neurons in Nucleus Beta of the Cat Inferior Olive Respond to Horizontal Rotation (Abstract). Society for Neuroscience Abstracts 10: 539, 1984.

Robinson, F., Hollerman, J., and Tomko, D. Cat Vestibular Neuron Responses to Active Head Rotations. Physiologist 28 (6, Suppl.): S147-S148, 1985.

Robinson F., Hollerman, J., and Tomko, D. Cat Vestibular Neurons That Exhibit Different Responses to Active and Passive Head Rotations (Abstract). Society for Neuroscience Abstracts 11: 321, 1985.

Tomko, D., Wall, C., and Robinson, F. Vertical Eye Movement and Vertical Canal Responses in Cat During Normal Pitch and During Pitch with the Animal on Its Side (Abstract). Society for Neuroscience Abstracts 10: 1153, 1984.

CHARACTERIZATION OF THE PROTEIN RESPONSIBLE FOR LATERAL AUXIN TRANSPORT DURING GRAVITROPISM AND THE ROLE OF PHOSPHORYLATION IN GRAVITROPIC ACTIVATION

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Description of Research

Lateral transport of auxin appears to be a key event among several gravistimulated processes thought to be involved in mediating gravitropism. In addition, polar or axial transport of auxin is a process closely linked to lateral transport and probably ultimately required for differential growth of cells in graviresponsive regions of plant shoots. An understanding of the molecular mechanisms of both polar and lateral auxin transport would undoubtedly contribute toward the elucidation of the molecular mechanisms which mediate gravitropism. This research is directed toward understanding these molecular mechanisms.

Virtually nothing is known about the biochemical processes that regulate auxin transport. A protein that may be part of a mechanism regulating auxin transport is the receptor which binds phytotropins. Phytotropins are artificial inhibitors of auxin transport and gravitropism; preliminary evidence for the existence of endogenous phytotropin-like compounds has been documented. The phytotropin receptor is closely associated with an auxin efflux carrier and is thought by some investigators to be the carrier itself. Other researchers, including myself, believe that it is a separate molecular species with regulatory properties.

The specific goals of this research are: (a) to test a general model (as proposed by B.G. Pickard) of shoot gravitropism which predicts an activation of lateral auxin transport via Ca^{2+} -calmodulin-stimulated phosphorylation of an auxin carrier, and (b) to test a specific hypothesis that phytotropin receptor binding activity is modulated by phosphorylation/dephosphorylation mechanisms and that a low binding activity-state of the receptor permits both polar and lateral auxin transport and gravicurvature, while a high binding activity-state restrains auxin transport and gravitropic curvature.

Purification of the phytotropin receptor is of great importance and the main objective of this research. Experiments with purified receptors should facilitate conclusive testing of these and other models.

Accomplishments

(1) Phytotropin receptor has been solubilized and partially purified (by conventional methods) from membrane preparations extracted from the graviresponsive region of the second internode of etiolated pea stems. As revealed by SDS-PAGE and silver staining, the partially purified preparation of solubilized receptors contains a maximum of about 25 polypeptides, the majority of which can only be visualized when loaded at 10X the concentration of the unsolubilized membrane protein. When equal amounts of protein are analyzed, the purified preparation contains 9 polypeptides; the unsolubilized membrane contains at least 120 polypeptides.

(2) Conditions for optimizing solubilization and for improving the stability of the receptor have been established. With the improved protocol 2X the amount of the receptor's binding activity is solubilized without a significant increase in the number of polypeptides. Additionally, with the improved protocol, 50 to 60% of the activity is retained after 50 hr storage as opposed to a 30 to 40% retention with the standard method.

(3) The receptor is sensitive to agents which inhibit phosphoprotein phosphatases and/or phospholipid phosphatases.

(4) Preliminary experiments indicate that the binding activity of the receptor is inhibited by sulfhydryl-directed reagents as well as serine residue-directed reagents.

(5) A ligand potentially useful for affinity chromatography purification of the receptor has been synthesized (synthesis was carried out by Dr. Terrence Riehl in the laboratory of Dr. Dabney Dixon) and applied in preliminary affinity chromatograph experiments. About 30% of the receptor binding activity appears to be bound to the affinity matrix but have not been recoverable to date.

Significance of the Accomplishments

Knowledge of the molecular and potential regulatory properties of the phytotropin receptor should further our understanding of auxin transport and of the events leading to gravitropic curvature. With a purified receptor, a more focused use of traditional biochemical methods will be possible and the introduction of modern molecular biological techniques to probe receptor function as it relates to auxin transport and potentially to gravity responses will be expedited. To this end, we have successfully completed several important steps leading toward the purification of the phytotropin receptor. Although our affinity chromatography system to date has not been perfected, the results obtained are encouraging. The effect of sulfhyldryl- and serine-directed reagents suggests that sulfhyldryl groups and serine residues are important for phytotropin binding to the receptor. Most importantly, indirect evidence has been obtained which suggests that phosphorylation/dephosphorylation mechanisms are linked to auxin transport systems. To our knowledge these preliminary findings are new in regard to phytotropin receptor research.

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16. Abstract This report consists of individual technical summaries of research projects of NASA's Space/Gravitational Biology Program, for research conducted during the period January 1986 to April 1987. This Program is concerned with using the unique characteristics of the space environment, particularly microgravity, as a tool to advance knowledge in the biological sciences; understanding how gravity has shaped and affected life on Earth; and understanding how the space environment affects both plant and animal species. The summaries for each project include a description of the research, a list of the accomplishments, and explanation of the significance of the accomplishments, and a list of publications.					
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